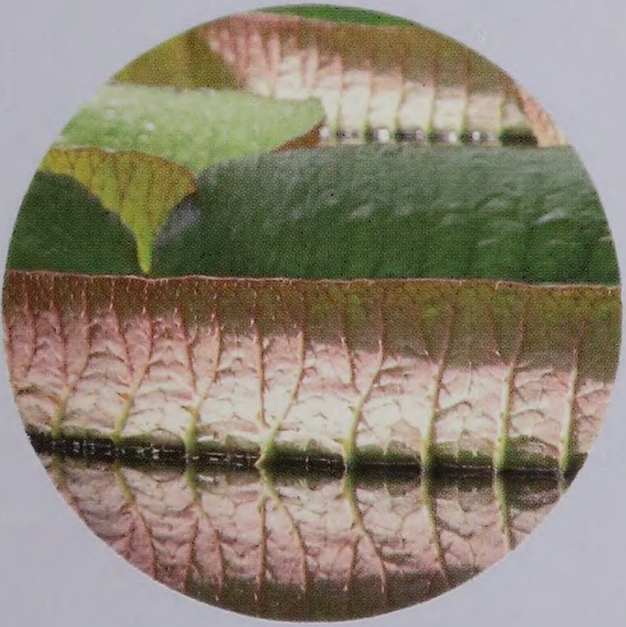
EUROGARD III PROCEEDINGS



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A. Bogaerts
(eds.)

EuroGard III

Papers from the
Third European Botanic Gardens Congress
and the
Second European Botanic Gardens
***Education Congress* (BEDUCO II)**

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Preface

The establishment in 1994 of a European Consortium to link the botanic gardens within the European Union was an extremely important step to enhance the role of European botanic gardens in Europe. The Consortium has played an important role by developing new cooperation in support of education and conservation of biodiversity and environmental cultural heritage in Europe amongst the botanic garden community. Over the ten years since its establishment the Consortium has been expanded to include representatives from each of the national botanic garden networks in the EU. It has provided an important medium and mechanism for the exchange of information, ideas and the results of practical experience between botanic gardens. Its activities have gone well beyond just providing a new forum for botanic gardens. Ever since its first meeting, held in Gran Canaria, Spain in May, 1994, it has worked to provide policy guidance and leadership for botanic gardens.

Some of the Consortium's most notable achievements have included (a) the preparation and publication of an *Action Plan for Botanic Gardens in the European Union* (published by the National Botanic Garden of Belgium in 2000), (b) ensuring that the role of botanic gardens was specifically included in the European Community's Biodiversity Strategy and (c) leading the development of the *International Plant Exchange Network* (IPEN) to respond to the access and benefit sharing provisions of the Convention on Biological Diversity.

The European Botanic Gardens Consortium has also organised a series of important and influential conferences for botanic gardens in Europe. These meetings have greatly assisted in the development of coherent responses to common concerns and for the development of shared policies amongst botanic gardens. To date three EuroGard Congresses have been held, in Edinburgh, Scotland, U.K. (1997), Gran Canaria, Spain (2000) and Meise, Belgium (2003), of which this volume represents the Proceedings. These meetings have also been important to assist in the exchange of ideas, to support the development of collaborative programmes and institutional links and to ensure that a botanic garden community is strengthened throughout Europe.

While the membership of the European Consortium itself is limited to representatives of botanic gardens networks within the EU, the EuroGard Congresses themselves have included participants from throughout Europe, helping to ensure that botanic gardens in Europe, north, south, east and west, work closely together to respond to the rapidly changing socio-economic environment in Europe and to address increasingly urgent environmental problems and issues.

The coming period will present new challenges for European botanic gardens, not only due to the expansion of the European Union to include ten more countries but also to respond effectively to challenges posed at national and international levels to the continued loss of biodiversity in Europe and throughout the world. At the *World Summit on Sustainable Development* (Johannesburg, 2002) the world community adopted a target to significantly reduce the rate of loss of biodiversity worldwide by 2010.

The European Union itself has now set itself an even more ambitious target for 2010, to halt the loss of biodiversity in Europe by 2010. It is clear that without the wholehearted involvement and participation of botanic gardens in national and local measures to conserve plants and natural habitats throughout Europe the chances of this target being achieved will be greatly reduced. It is therefore very significant that at the EuroGard Congress III in Meise, delegates reaffirmed their commitment to the implementation of the *Action Plan for Botanic Gardens in the European Union* and proposed the development of a series of outcome-orientated targets, milestones and indicators to help monitor and promote the implementation of the Action Plan (see the Resolutions from EuroGard III Congress in this volume). Such targets will also be of considerable importance to align botanic gardens more closely with the European-wide measures being taken to achieve the global 2010 target of biodiversity loss; to achieve the objectives of the *Global Strategy for Plant Conservation* (adopted by the Convention on Biological Diversity in 2002) and to ensure that we make our effective contribution to the National Biodiversity Strategies and Action Plans in Europe.

This volume includes a wide range of articles from botanic garden contributors throughout Europe, representing a series of the presentations delivered at the Congress. The contributions are extremely diverse and provide a valuable new resource and permanent record of good practice, models and case studies for botanic gardens to use to enhance their own activities. Practical issues are addressed by articles which focus on subjects including the development of electronic databases and information systems to the creation of nurseries, seed banks and other botanic garden facilities. Reviews of innovative model activities in botanic garden research, education and conservation programmes are also presented. Several contributions also provide fascinating insights into the results of botanic garden scientific research on plants of Europe and beyond. It is clear from even the most cursory glance at this useful volume that the importance of European botanic gardens is enormous, reflecting their remarkable diversity, wide resources and extensive programmes.

I congratulate the Director and staff of National Botanic Garden of Belgium not only for their support in hosting the EuroGard III Congress in July 2003, but also for preparing and publishing these Proceedings. I hope that botanic garden staff and those who work with botanic gardens throughout Europe will use this book to stimulate and support new activities within and beyond botanic gardens.

Botanic gardens are clear in their understanding that their work must support the European environment, serve local communities and respond to national concerns and priorities. Milestone events such as the EuroGard Congresses provide an effective means to ensure that botanic gardens in Europe are not only important scientific institutions but also underpin the developing movement for increasingly effective management of our unique European plant resources and increased public awareness of the importance of plants, their wild habitats and our fundamental reliance on plants for life.

Peter Wyse Jackson
Secretary General
Botanic Gardens Conservation International, U.K. [www.bgci.org]

13th May, 2004

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Atlantis-BG, a standard for comprehensive, web-based collection management

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Abstract. *– Atlantis-BG is a state-of-the-art programme that links the plant collection database of a botanical garden to other relevant scientific data, such as literature, authors, collectors, donors and illustrations. It also contains address information of relations such as gardens, donors, contact persons etc. Atlantis-BG connects important information sources, available on the world wide web, and maintained by different organisations, such as the collection data of other botanic gardens. This means that these information sources are kept up-to-date not by the garden but by the organisation that makes that information available on the web. Atlantis-BG supports taxonomic research by linking synonyms to the accepted name, and also allows homonyms. Scientific data linked to synonyms viz. different ranges for synonym names as for the accepted name, as is often the case, can also be maintained. Taxonomic data are presented in a tree of taxon records with an infinite number of ranks possible, each rank with its own data. For each rank it can be chosen to show this level and its synonyms or keep them hidden. Atlantis-BG supports global standards such as the ITF2-format and WGS-origin-codes (including Gazetteer) as well as author abbreviations. Handling of internet requests for seeds e.g. can be processed automatically and seed lists can be generated in html-format and published on the web within seconds, including images. Atlantis-BG has both a multi-user windows interface as a web based interface. It includes full authorization handling for different types/levels of data and an easy to use seed list and seed management tool as well as garden-location event management tools. All kind of electronic data can be stored, documented and linked to Taxa, Accessions, Relations etc. including electronic seed lists, images and articles. Atlantis-BG is a new development in the information technology supporting scientific collection management. It incorporates the new advantages of the web as well as GIS. An overall-shell will be developed so that the databases of the several gardens appear as one national (European ?, global ?) database.*

Introduction

A new powerful tool called Atlantis-BG has been developed for and together with the Dutch University gardens of Utrecht, Amsterdam, Delft, Leiden, and Wageningen by DEVENTit Multimedia BV, Bunschoten, The Netherlands.

The development of standards concerning collection management databases for botanical gardens in The Netherlands started in 1984 (Aleva & al. 1984). Dutch Botanical gardens have been working with collection management databases since 1987. In spite of agreed functional standards, different technical solutions were used by different botanical gardens. All solutions were character oriented and covered only a part of the necessary processes. For example for

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literature, relation and seed management separate databases were used, all character oriented, or even lists on paper or in books, for example for author abbreviations (Brummitt & Powell 1992).

At the end of the Nineties, thanks to governmental foundations, as a part of a national project aiming the disclosure and digitalisation of academic collections in The Netherlands, means were found to obtain a new database for the five Universal Botanical Gardens mentioned above. A common database should make exchange of information a lot easier and a uniform presentation of (National) collections on the internet possible. It should also combine all sort of small databases used into an all-containing one, and should disclose localities of collection elements by means of a GIS-like functionality.

In the decision process whether to obtain an existing standardised database (such as BG-base or SysTax both of which were looked at thoroughly) or to develop a completely new one, the choice was made for a new one because it was agreed that the advantages of a development with the newest technologies such as an object-orientated database (Martin 1993; Booch & al. 1998) which can include all sorts of multimedia objects and a standard web (and windows) -interface weighed up to the disadvantages of starting something new and unknown while a widely accepted relational database such as BG-base, although older and many times upgraded, is already used (still often in the DOS-version) by many Botanical Gardens in the world.

A combination of technical knowledge and knowledge of the demands of collection management and the structure of its database were found in the corporation of the curator of the Utrecht Botanical garden (drs. E.J.Gouda) with a software-house specialised in the development of software of collections and archives in general. It took three years of development and testing to accomplish the first version of the database.

The newly developed application "Atlantis Botanic Garden" is a standard application on the Atlantis platform, which is developed specifically for museums and archives. All applications of this platform have functionality in common, such as an object oriented database for storing images and other multi media information, a reporting option, and a super fast full text search engine with synonym and phonetic search capacity for finding information in the database. However, every Atlantis program is specifically designed for its own particular type of collection.

Description

A database for collection management in botanical gardens should support all kinds of users, such as scientists, curators, students, gardeners, the educational staff, etc., so an authorisation feature is added.

For the public a transparent web interface is developed, including search possibilities on several levels such as accession and taxon. All objects and their properties can be shown on the web interface including the multimedia objects as photographs, articles, maps etc.

The main level structure is as one would expect for botanical gardens and consists of four levels. The highest level is the level of the taxon, followed by accession, accession location and event. Around this kernel of four levels are all other structural elements.

The screen is divided into two parts, the left one being a tree of interlinked objects and records, the right one containing the information, often in more than 1 property sheets, of the selected object in the tree on the left.

There are three main entry buttons. One gives the taxon tree, one gives the accession, accession-location and event trees, and one gives all other relevant trees of the database such as relations and persons, authors, literature, WGS-system and so on.

Data structure

Taxon. On taxon level it is possible to display the whole taxonomic system as a tree (31 ranks are possible, from Regnum to forma and hybrids) and using drag and drop features can easily make changes in taxonomy. I.e. a genus with all species belonging to it can be hung under a different family in one action. This can be done on all levels. The parent taxon (or taxa, in case of hybrids) of a taxon determines its place in the tree. Correct names are bold, synonyms are not, so they are easily distinguishable. Higher taxonomical levels can be left out of the tree for transparency's sake. The number of living accessions are indicated in the tree. Each garden can choose for its own taxonomy tree, or can choose to follow a standard [such as Cronquist (1981) or APG (Judd & al. 1999)] or an alternative.

On the right side of the screen information on a specific taxon is displayed in six property sheets one of which contains multimedia objects. A multimedia object can be a picture or a distribution map or a film or an article on that taxon. The first property sheet contains a button to enter the accessions linked to this taxon. Another property sheet gives access to all relevant literature in the database to be attached to the taxon. On the other hand one literature reference has links to all relevant taxa and accessions. A direct link can be made to sites on the internet like IPNI, IUCN, IOPI, USDA, FL_EU etc. for a direct check of the name and an indication of its distribution. Standard taxon use categories can be applied including geographical information.

Accession. An accession is a plant or group of plants of one taxon, originating from the same source (e.g. seeds from one seed list number, plant, cutting, etc.) and obtained at the same time. An accession may be found in the garden at more than one location.

The screen structure is the same as in taxon. On the left side is the accession tree. Branches show the identifications and verifications, information about where the accession is located (the accession location) in the garden and information about derived material, such as seeds and herbarium specimens and seed list information. The property sheets on the right side of the screen contain all relevant information on an accession, such as source, donor, agreement notes, propagation etc., including a property sheet for multimedia objects for that accession. Standard ITF2 abbreviations (Jackson 1998) are used for all relevant fields on an accession.

When searching for a certain accession there is an option to run the search criteria over any text in the accession part of the database or over text within specified fields. This can be done in any specified tree. For each accession in the tree, there is an indication of its status (current or not). Each verification for an accession, and other relevant information such as the date, the name of the verifier and the level of identification can be recorded. The most recent one will be the accepted name for this accession.

Accession location. On this level specific information of individual plants or a group of plants is given including the location in the garden where it can be found. Coordinates can be added from GPS-measurements and maps of the garden with selected accession numbers drawn in it can be processed. In the illustration property sheet in Garden location maps can be added in different formats and by means of two reference coordinates these maps can be synchronized to the attached coordinates of each accession, so that in the mapping report the position of the plant is printed on the right place of the map. One accession location can be linked to more events.

Event. An Event occurs to an accession at a certain location, for example sowing, germinating, flowering and dying. The users are free to define Event types themselves. One can select one or more accession locations and fill in an event that is common to all selected accession locations. For example the event germinated can occur for more accession locations on one day. This feature can be used by the head gardener on location.
Free fields. User-defined fields can be imported in a special screen at the accessions and taxa, for example for horticultural information.

Relations. In the tree 'Relations' names and addresses of relations, such as companies, colleague-gardens, etc. can be inserted. Atlantis-BG has a mail merge facility for mailing to a number of selected relations. E-mail addresses and web-sites can be recorded as well, and Atlantis-BG can send e-mails to relations or connect with a web-site.

WGS system. A geographical standard WGS-tree can be added (Hollis & Brummitt 1992), including a gazetteer to find localities.

Processes

Reports. Reports can be made using XML ruler templates producing HTML output. Within these templates different criteria, sorting priorities, fields, etc. can be chosen. Reports can be drawn up for each desired object and can include images, maps, www links etc.

Labels. For the production of labels of relations or plants mail-merge for MS Word is used. Any selection can be made and any field, size and lay-out chosen. It is also possible to use reports instead.

Seed list. A seed lists can be generated directly from the database as a report. It can also be exported in ITF2 format. The seed list may be composed in HTML format, allowing the user to publish the seed list via Internet. In this seed list, illustrations (for example photographs in .jpg) of the accessions or taxa can be inserted. Available amounts and seed orders from other gardens can be inserted, and at sending time the database can process all the relevant labels (gardens and accessions) in the right order. In this way the whole seed list process is covered.

Living collection count. It is possible to generate a count report of the whole, or part (one family or genus for example) of the collection including number of genera, number of species, number of cultivar's and number of indet's. Determined and undetermined accessions are counted separately, as well as wild and cultivated origins. This gives a good indication of the quality of the collection.

Import and export. Data can be imported and/or exported in ITF2 format, making exchange of data possible.

Conclusions

Development of Atlantis-BG is never finished, since new demands and possibilities on collection management in botanical gardens will rise. Development will continue. Atlantis has already proved to be a database with many possibilities. It speeds up the routine-parts of the administration process of collection management and uses the present communication possibilities very efficiently. It makes it possible to cope with changing ideas in taxonomy. The disclosure of botanical collections to the public forces curators to work away any possible backlogs in administration as quickly as possible. This is a great challenge as a result of the challenge of creating a completely new database.

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Vilnius University Botanical Garden at work

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Abstract. *– In this paper the Botanical Garden of Vilnius University is presented. The main activities are the following: conservation of plant genetic resources, scientific research, training and education. At the moment there are seven departments. In the collections of the Botanical Garden of Vilnius University 9000 taxa plants of 190 families and 866 genera are grown. Recently the garden is involved in the programmes of plant genefund research and protection, other scientific and education projects etc.*

Key words: *Botanical Garden of Vilnius University, plant, genetic resources, scientific programmes, projects.*

Introduction

The Botanical Garden of Vilnius University was founded in 1781 by a French professor, J.E.Gilibert. At its first site the Garden (now Pilies St. 22 in the Old Town of Vilnius) covered 300 square meters where 2000 species of plants were grown. From 1784 till 1787 the Garden was headed by a famous German traveller and scholar G.Forster. In the first half of the 19th century the garden was led by professor S.B.Jundzill. In 1827 8979 plant taxa were grown. Following the Lithuanian-Polish rebellion, the Russian government closed down the Botanical Garden in 1841. In 1920 only the Botanical Garden was reopened by Polish botanists. At start the Garden consisted of an area of 2 hectares and expanded in 1962 to 7.35 hectares. In 1974 an additional 150 hectares in Kairenai, in the north-eastern part of Vilnius were allocated to the Botanical Garden. At the moment the Laboratory of the Plant Biochemical and Physiological Research is housed there as well as several other departments: Plant Genetics, Dendrology, Floriculture, Pomology and Plant Systematics and Geography (Zilinskaite 1999). The staff includes 6 doctors, 28 specialists and 50 workers.

Material and methods

The main activities of the Botanical Garden are conservation of plant genetic resources, scientific research, training and education. The scientists of the Garden are working in four scientific programmes. Today the Botanical Garden is striving to be not only a research centre, but also to become an attractive recreation site for the population. For this purpose five projects for the

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development of the garden infrastructure have been carried out. A new project for education is planned.

Results

The conservation of Plant Genetic Resources and Plant collections. Vilnius University Botanical Garden takes part in the national programme "Genetic Resources" which was started in 1998 by the Lithuanian Ministry of Education and Science. The programme encompasses the conservation and research of the genetic resources of agricultural and horticultural crops, medicinal and ornamental plants, forest trees and domesticated animals. The main work on genetic resources is carried out in five departments of the Botanical Garden:

1. The Department of Dendrology. The collection contains 2460 taxa (species, subspecies and cultivars) belonging to 229 genera, 79 families. 1200 species from different geographical regions: Europe, Far-East Asia (China, Japan), North America, Siberia, the Mediterranean. The woody plant collection of about 500 taxa is the only one in Lithuania (Skridaila & al. 2001). Main collections: *Ericaceae* Juss. - 458 taxa, (including *Rhododendron* L. - 260), *Syringa* L. - 160, *Rosa* L. - 100, *Juniperus* L. - 120, *Clematis* L. - 61 (most species) etc. (Naujalis & Skridaila 2001). Activities: investigation of woody plants in old manor parks, forests and other green areas of Lithuania, research work in the department's collections, and conservation. Website: http://www.dendrologia-lithuaniae.lt
2. The Department of Plant Genetics. The collection of induced and natural mutants of cereal crops is maintained and investigated only at Vilnius University. The department has natural mutants (about 80 species), but the most important is the original collection of induced barley mutants and revertants (about 300 collection numbers). It includes several genetic unstable loci: *be* (branched ear), *tw* (tweaky spike) and others (fig. 1). The collection of barley also has genetic stocks, cultivars and mutants with marker genes and genes which are determining a high resistance to fungi infection or a high lysine content. There are 34 field beans mutants.

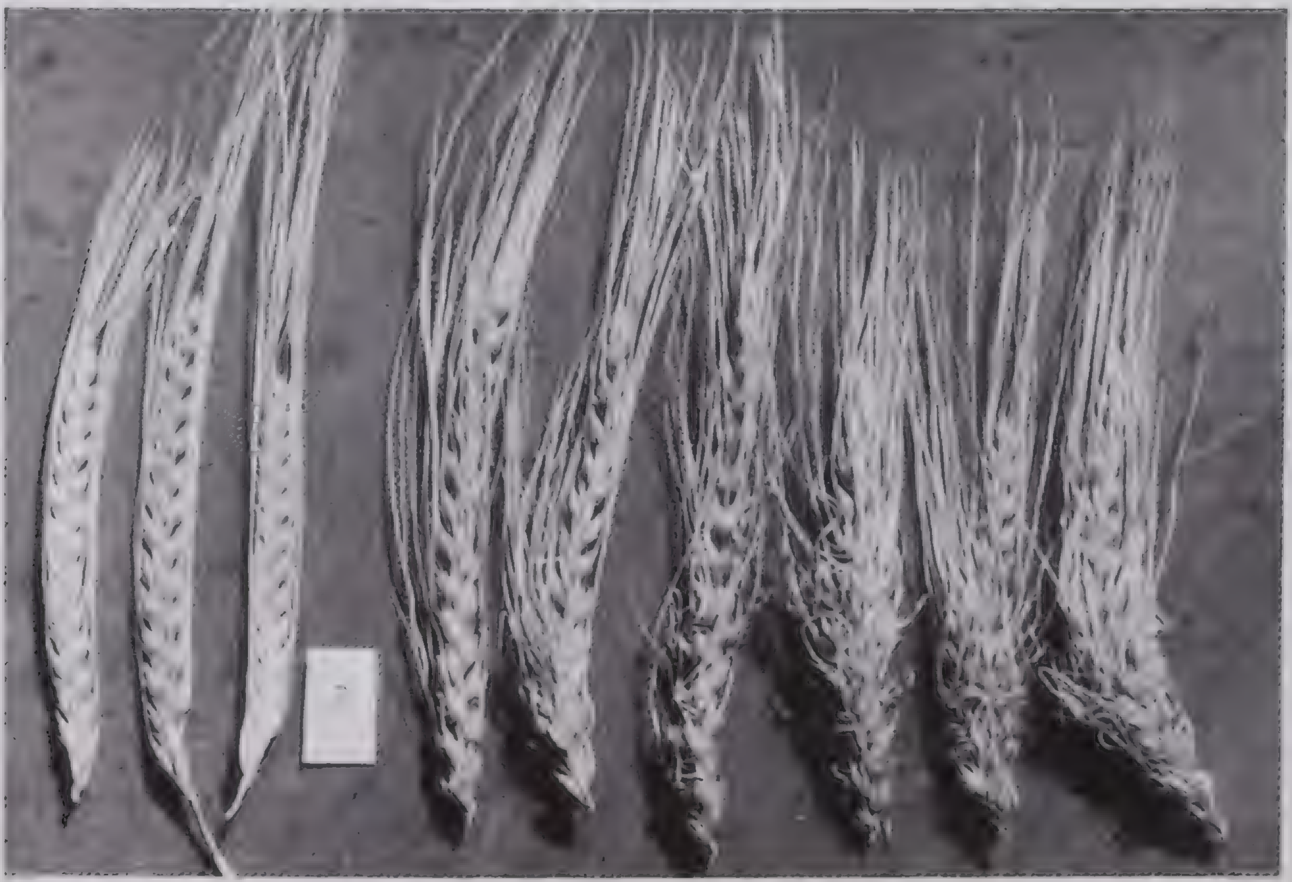

Figure 1. Barley mutants with genetic unstable loci: tw (tweaky spike) and be (branched ear).

3. The Department of Floriculture. The collection contains more than 3200 taxa of ornamental herbaceous plants. It has unique genetic resources of Lithuanian origin (table 1). Main activities of the department: introduction and investigation of ornamental herbaceous plants and accumulation and preservation Lithuanian hybrids of herbaceous ornamental plants (Dainauskaite & al. 2001).

Table 1. The main collections of the Floriculture Department.

Genera	Number of taxa		
	Introduced cultivars	Lithuanian cultivars	Total
Aster L.	90	0	90
Astilbe Buch.-Ham. ex D.Don	70	0	70
Chrysanthemum L.	5	0	5
Crocus L.	42	18	60
Dahlia Cav.	310	170	480
Gypsophila L.	3	0	3
Gladiolus L.	30	140	170
Hemerocallis L.	77	3	80
Hosta Tratt.	20	0	20
Iris L.	360	200	560
Lilium L.	65	70	135
Narcissus L.	145	95	240
Paeonia L.	167	91	258
Phlox L.	90	0	90
Primula L.,	50	40	90
Tulipa L.	60	270	330

4. The Department of Pomology. The collections contain plants of 832 taxa. The main collections: *Ribes* L. - 493 taxa, *Vitis* L. - 113, *Lonicera* L. - 72, *Vaccinium* L. - 47, *Sorbus* L. - 56. The oldest and most abundant collection of horticultural plants are the currant and gooseberry collections. The collection includes 86 Lithuanian A. Gailiunas grape clones, 37 Lithuanian honeysuckle clones, 200 quince collection numbers.

5. The Laboratory of Plant Physiology and Biochemistry. There are 111 species and varieties of herbaceous and woody plants belonging to 16 genera in the collection *in vitro* (Dapkuniene & al. 2002).

6. The Department of Plant Systematics and Geography collections. The collections of the department contain plants of 2800 taxa. The main collections: systematic collection of plants - 700 taxa, greenhouse plants - 690, ornamental herbaceous plants - 1000, woody plants - 400 (200 of them roses).

Scientific work. In 1998 scientists of Botanical garden joined the programme of investigation and protection of genetic resources. Since 2002 the Ornamental Plants' Genefund Resources Research and the Protection Coordination Centre is working in the Botanical Garden of Vilnius University. Since 2002 a central European data registration base for *Ribes* L. and *Rubus* L. genera has been created under authorisation of IPGRI (Italy). Website http://www.ribes-rubus.gf.vu.lt. Scientists of the Garden are involved in the programmes "Control of physiological process of plants by means solid-state lighting" (HORTILED) and research on *Rubus* L. populations (together with Nordic Gene Bank).

Education. The Botanical Garden is an institution which is involved not only in research, but also in educational activities. The interest of the public in the Garden has grown significantly in recent years. About 200 students of universities and Vilnius Colleges have been using plant collections of the Botanical Garden for learning purposes. For this reason every year 3000 schoolchildren and about 100 biology teachers visit the garden. A new project for education is planned too.

Infrastructure. At the end of 2002 it was decided to review and renew the Botanical Garden development plans, taking into account new opportunities and new social and economic conditions. One of the Botanical Garden strategic aims is to turn Kairenai into a modern centre of science, studies, education, recreation and tourism. It should become a National Botanical Garden and meet a wide range of needs of the public. To achieve this it has been planned to attract both private investors and different public organisations as partners. A multilateral agreement between the Ministries of Environment, Culture, and Education and Science and the Vilnius City Municipality is being negotiated. Discussions with private entrepreneurs are underway regarding four projects: establishing golf-links and an aquarium, building a camping site, organising horse riding services. One international project with Japanese partners has been started; a project for state capital investment for 2004 has been prepared.

In 1999–2002 0.25 million euro were spent on the development of the garden infrastructure. In 2004-2006 it is planned to accomplish works for an additional 1.7 million euro. In the autumn of 2002 both the building of the main entrance and the parking was begun, new plant expositions were established to make the Garden more attractive for the public. Events in the Botanical Garden are very popular and are attended by hundreds of visitors. As a result of the successful implementation of the projects the number of visitors in Botanical Garden is expected to increase tenfold – up to 100000 per year. The Botanical Garden environment, its rich heritage, natural and social aspects have a great potential.

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The development of a native plant collection nursery in the Kroussia Balkan Botanic Garden

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Abstract. – *The Balkan Botanic Garden situated at Kroussia mountains and the Balkan Horticultural Research Institute in Thermi/Thessaloniki aspire to become a major scientific and educational center for the Mediterranean and Balkan Flora. Until now, the Garden's physical infrastructure has been established, the national living plant collection has been initiated and the groundwork for cooperation with neighbour Balkan and other countries has been laid-out. Its basic mission is to conserve and promote the Greek and Balkan flora. All plants in the Garden derive from plant material grown at the nursery located in the installations in Thessaloniki. Among the native species maintained at the Garden three major groups can be distinguished: annuals, perennials and perennial herbs. Special consideration has been given to the research on the perennial herbs and annuals because of their characteristics, which make them ideal plants for landscape use under temperate climates. In addition, perennial herb species could play a significant role in pharmaceutical industry. This study is concentrated on their propagation. Results collected from different treatments during the past five years proved that annuals could be propagated without problems using well preserved seeds collected from mother plants, while vegetative propagation is the most appropriate method for perennial herbs, as the need for maintaining their genotypes and characteristics is the main priority for these species. Propagation protocols have been established.*

1 Introduction

Balkan Botanic Garden Kroussia (BBGK) is a newly established garden in Greece. It occupies an area of 31 hectares and it is located at the Prefecture of Kilkis. The Garden houses native Balkan species into natural ecosystems, representing the five Greek and Balkan vegetation zones. Different plant environments of machi and bushwoods, of grasslands and fields with bulb and graminaceous plants, of alpine and sub-alpine vegetation, of aromatic and pharmaceutical plants, of perennials, of aquatic and hydrophilic plants as well as arboretum elements have been established, taking into consideration taxonomic relationships, ecological adaptations and different life histories of the endemic species.

Conservation, propagation and assessment of native plant species, endemic, rare and threatened, as well as education and consultation of aspects concerning the native Balkan Flora is taking place at the Center of Research and Education located in the city of Thessaloniki.

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The objectives of the BBGK are multiple and lasting: **conservation, preservation** and **management** of native Balkan Flora, especially of rare and threatened species, **establishment** of interregional cooperation with the Balkan countries and **boost** of the borderlands via tourist enhancement and development of alternative forms of avocations, **education** of various target groups and public awareness concerning conservation of natural environment.

The present paper aims at reporting on five years of applied research on appropriate propagation methods for different native species. The results lead to propagation protocols of annual and perennial herbs, described for the first time.

2 Materials and methods

2.1 Plant collection expeditions

All native plants maintained at the nursery derive from natural populations. Collecting expeditions are conducted based on the floristic exploration of the area and the recognition of its floristic wealth (e.g. National Park, inclusion in the NATURA2000 network of protected sites, etc.). For each plant, plant part or seeds collected a collection sheet includes accession number, geographical data, altitude, latitude and longitude, and habitat description. Plants collected from nature receive immediate care in the nursery. They are automatically designated as stock plants, planted in large containers or special places according to their needs, and are given special treatments since they recover from the transplantation shock.

2.2 The Botanic Database

In the Botanic Database all relevant data of the collected plants are recorded (see table 1).

Table 1. Data comprised in the Botanic Database

1. Asn
2. Collecting site
3. Quantity
4. Mother plants
5. Photograph
6. Herbarium
7. Endangered species
8. Plant type
9. Flower colour
10. Flowering period (bibliography)
11. Flowering period (open air)
12. Flowering period (greenhouse)
13. Family
14. Genus
15. Species
16. Author 1
17. subspecies
18. Author 2
19. Vernacular name (Greek)
20. Vernacular name (English)
21. Vernacular name (German)
22. Cultivar name
23. Collector's name
24. Verifier's name25. Institute
26. Date
27. Country
28. State
29. Specific geographical site
30. Nearest village (Km)
31. Altitude
32. Latitude
33. Longitude
34. Habitat description
35. Bibliographic description of the habitat
36. Remarks

2.3 Propagation methods

Applied research on propagation methods were market driven having as objective to provide the interested target groups with propagation protocols of annual and perennial herbs.

2.3.1 Annual plants. Sexual propagation of six annual native plants, *Consolida ajacis* (Ranunculaceae), *Chaerophyllum coloratum* (Apiaceae), *Asphodelus fistulosus* (Liliaceae), *Malcolmia flexuosa* (Brassicaceae), *Delphinium staphisagria* (Ranuculaceae) and *Nigella damascena* (Ranunculaceae) was examined. Seeds, harvested from the collection of mother plants, during summer (June, July) or autumn (September, October) were used. Storage period was the maximum 2 months in glass vessels containing silica gel at room temperature 10-20 °C. Seeds were sowed in a medium of peat (Klasmann, KTS 1): perlite:soil 2:2:1 v/v in propagation trays. The trays were placed on benches inside a heated plastic greenhouse and they were irrigated properly according to the needs of the medium and the climatic conditions.

Seedlings were transplanted after their germination in pots of 2.5 l in a substrate of peat (Klasmann, KTS 2): perlite : soil (2:1:2 v/v). Fertilization treatments with balanced fertilizer (20-20-20) were applicated every 15 days, one month after germination. Irrigation took place two or three times per week depending on the climatic conditions. After transplanting, the plants were placed outside the greenhouse under shading frame (50%) and finally some days under direct sun.

2.4.2 Perennial herbs. The most appropriate asexual method of propagation was examined for the Lamiaceae *Origanum dictamus, Salvia officinalis* and *Teucrium halacsyanum*, endemic of Greece, and *Lavandula angustifolia, Rosmarinus officinalis* and *Thymus mastichina*, plants very used in landscaping and in the pharmaceutical and perennial herbs industry. Softwood stem cuttings of one or two nodes were taken from mother plants during early spring. After they were treated with indole-3-butyric acid (IBA) applied as powder (0,066% v/v) they were placed in a substrate of peat (Klasmann, KTS 1) and perlite (1:3 v/v) or in pure perlite in propagation trays. The trays were placed at bottom heat benches in a plastic greenhouse. Soil temperature was kept at 21°C, while air temperature was 18-25°C depending on weather conditions. Relative humidity was maintained between 70-85% by mist. After rooting, plants were gradually acclimatized reducing the RH 20% every week. When rooting system was developed enough and plants were acclimatized, they were transplanted in pots of 2.5 l in a substrate of peat (Klasmann, KTS 2): perlite 3:1 v/v. Potted plants were placed outside the greenhouse under shading frame (50%) and finally some days under direct sun.

3 Results and discussion

3.1 Living plant collections and dried specimens

The flora of Greece, consisting of approximately 5700 species of higher plants, is one of the richest in Europe in proportion to its area. It is estimated that about 13% of the plant taxa are endemic. Many endemic plants are found in habitats, which are naturally fragmented, such as mountain tops, cliffs, sand dunes and islands. According to the Red Data Book of Rare and Threatened Plants of Greece 263 taxa are considered rare and threatened (Phitos et al. 1995).

More than 50 collection expeditions since 1997 resulted in the establishment of a mother plant collection at the nursery, comprised by 849 native Balkan acquisitions from which 408 are fully named to species, while the rest remains only partly identified. The main categories which are preserved in our native plant nursery are annuals, perennials and perennial herbs. Six examples of annual plants from the area are enumerated in section 2.3.1. Table 2 shows endemic species characteristic of the area. These plants have not been cultivated before, so the nursery was developed from a specialized propagation unit. The goal is to propagate all plants collected from their natural environment.

Research conducted regarding propagation and uses is mostly concerns annual and perennial herbs. Their unique characteristics make them ideal plants for landscape use under temperate climates. In addition perennial herbs play a significant role in pharmaceutical industry. The use of local flora for plant cures is in fact inherent to rural societies. However, only a limited number of medicinal plants are being cultivated (Arora & Engels 1992).

Dried specimens are also maintained. For each accession there is a specific record, which is the base for identification, done in cooperation with the Herbarium of the Botanic Department of Aristotle University of Thessaloniki.

3.2 Annual plants

Results derived from seed germination efforts varied depending on the species and on storage period (table 4). *Consolida ajacis* and *Malcolmia flexuosa* gave high germination percentage, 91% and 69.7% and the young plants were ready to be transplanted after 30 and 50 days respectively. *Asphodelus fistulosus* and *Chaerophyllum coloratum* resulted in moderate germination percentage of 55% and 37%, while *Delphinium staphisagria* and *Nigella damascena* showed limited germination 18% and 10% respectively.

Primary factors influencing seed longevity are moisture content, storage temperatures and seed quality at the time of initial storage (Stanwood 1987). Long term preservation storage conditions recommended by the International Board for Plant Genetic Resources (Cromarty & al. 1982: 2-3) for most seeds are 18 °C and 4-7% seed moisture content in sealed containers. Blocks of germination in seeds allow time for dispersal and increase longevity. These blocks may take the form of seed coats, impermeable to water, inner membranes impermeable to oxygen, rudimentary embryos, chemical inhibitors, hormonal and chemical processes in the embryo and endosperm or combinations of these qualities. Certain treat methods should be applied aiming in uniform germination (Atwater & Vivrette 1987).

Table 2. Endemic perennial plants at the living plant collection of BBGK.

Brassicaceae	*Aubrieta thessala*
Brassicaceae	*Rorripa thracica*
Caryophyllaceae	*Cerastium decalvans*
Caryophyllaceae	*Dianthus corymbosus*
Caryophyllaceae	*Dianthus gracilis*
Caryophyllaceae	*Paronychia rechingeri*
Caryophyllaceae	*Silene fabarioides*
Caryophyllaceae	*Silene flavescens*
Compositae	*Achillea ageratifolia*
Compositae	*Achillea ambrosiaca*
Compositae	*Achillea grandifolia*
Compositae	*Achillea holosericea*
Compositae	*Centaurea napulifera*
Compositae	*Lactuca intricata*
Compositae	*Senecio squalidus*
Compositae	*Staechelina uniflosculosa*
Crassulaceae	*Jovibarba heuffelii*
Euphorbiaceae	*Euphorbia cyparrissias*
Fabaceae	*Ebenus cretica*
Fabaceae	*Genista radiata*
Geraniaceae	*Geranium macrorrhizum*
Gesneriaceae	*Haberlea rhodopensis*
Gesneriaceae	*Ramonda nathaliae*
Iridaceae	*Iris reichenbachii*
Juncaceae	*Juncus thomasii*
Linaceae	*Linum elegans*
Plumbaginaceae	*Armeria rumelica*
Rosaceae	*Potentilla detommasii*
Saxifragaceae	*Saxifraga spruneri*
Scrophulariaceae	*Digitalis lanata*
Scrophulariaceae	*Odontites linkii*

Table 3. Perennial herbs at the living plant collection of BBGK.

Cistaceae	*Cistus creticus, monspeliensis*
Compositae	*Achillea millefolium*
Lamiaceae	*Acinos alpinus, majoranifolius*
Lamiaceae	*Ballota acetabulosa*
Lamiaceae	*Calamintha nepeta*
Lamiaceae	*Lamium garganicum*
Lamiaceae	*Lavandula angustifolia, stoechas*
Lamiaceae	*Melissa officinalis*
Lamiaceae	*Mentha aquatica, crasnodorscaia, longifolia, microphylla, piperita, pulegium, spicata*
Lamiaceae	*Micromeria juliana*
Lamiaceae	*Nepeta maery, nuda, spruneri*
Lamiaceae	*Origanum dictamnus, majorana, vulgare*
Lamiaceae	*Phlomis floccosa, lanata, samia*
Lamiaceae	*Rosmarinus officinalis*
Lamiaceae	*Salvia officinalis, brachyodon, glutinosa, sclarea, verticillata, willeana*
Lamiaceae	*Satureja calamintha, horvati, montana, thymbra, pilosa, vulgaris*
Lamiaceae	*Teucrium arduini, chamaedrys, divaricatum, flavum, halacsyanum, polium*
Lamiaceae	*Thymus atticus, boissieri, bracteosus*
Lamiaceae	*Corydothymus capitatus*
Lamiaceae	*Thymus longicaulis, mastichina, sibthorpii*
Valerianaceae	*Centranthus ruber*
Vitaceae	*Vitex agnus-castus*

Research on these native species is in an initial phase as experience or references are missing. Moderate or limited germination of *Asphodelus fistulosus* and *Delphinium staphisagria* was predictable due to the short storage period between seed collection and sowing date (table 4). Low germination percentage of *Nigella damascena* could have another cause since the storage period was 5 months, long enough for dormancy break in annual species, and could be attributed to many parameters such as unsuitable storage method or pre-treatment of seed before germination procedure.

Table 4. Annual plants: sexual propagation by seeds

Species	Collection of seed	Storage period (months)	Seed germination (days)	No. of seeds / g	Germination success (%)	Transplanting period (days after sowing)
Consolida ajacis	June	5	27	379	97	30
Chaerophyllum coloratum	July	4	12	204	37	50
Asphodelus fistulosus	September	1	36	328	55	30
Malcolmia flexuosa	June	5	6	2346	69.7	30
Delphinium staphisagria	October	1	40	28	18	40
Nigella damascena	June	5	40	300	10	60

Table 5. Perennial herbs: asexual propagation by cuttings.

Species	Medium	Rooting (%)	Rooting period (days)	Transplanting period (days after rooting and
Lavandula angustifolia	Perlite:Peat	78.6	20	40
	Perlite	77.3	20	40
Rosmarinus officinalis	Perlite:Peat	96.1	15	30
	Perlite	65.0	15	30
Thymus mastichina	Perlite:Peat	14.8	25	50
	Perlite	59.5	25	50
Origanum dictamnus	Perlite:Peat	93.6	15	30
	Perlite	44.2	15	30
Salvia officinalis	Perlite:Peat	58.3	30	50
	Perlite	79.4	30	50
Teucrium halacsyanum	Perlite:Peat	49.2	30	50
	Perlite	80.0	30	50

3.3 Perennial herbs

Lavandula angustifolia showed no difference in rooting between two substrates mixture tested (78.6 and 77.29 %). *Rosmarinus officinalis* and *Origanum dictamus* showed higher results in perlite:peat, 96.10 and 93.55 % respectively than in pure perlite (64.97 and 44.19%). *Thymus mastichina, Salvia officinalis* and *Teucrium halacsyanum* gave higher rooting success using pure perlite as substrate medium, 59.45, 79.40 and 80.00% respectively, while the use of perlite:peat resulted in respectively 14.81, 58.33 and 49.20% rooting success (table 5).

After minimum 40 days, depending upon the species, young plants have been transplanted and continue to grow. Usual cultivation treatments were given such as fertilization with balanced fertilizer 20-20-20 with micronutrients. *Teucrium halacsyanum* showed a problem related in iron deficiency and additional treatments with iron fertilizer (Sequestrene 80) have been applied.

The Greek flora is one of the richest in the world concerning aromatic and pharmaceutical plants (Papapanagiotou *et al.* 2001). Unique species and special climatic conditions make the Greek and Balkan area ideal for the selection of this category of plants, rich in essential oils. These species are highly demanded for use in landscaping, in the pharmaceutical and aromatic industry, and for special purposes such as biological control of pests (Thanasoulopolos & Gianna 1997). The market needs certain selected biotypes rich in concentration of essential oils (Grigoratos 2002).

Since characteristics of the species must be retained and performance of genotype strongly depends on the starting material (Pank 2002) sexual propagation is out of the question for these plants, even though some of them present high germination success. Propagation using new tissue culture techniques has not been very successful due to problems related to the high concentration of essential oils and phenolic compounds in aromatic and pharmaceutical species (Curtis & Shetty 1996). Thus the most appropriate and economical propagation method seems to be the rooting of softwood cuttings as it has been estimated for other, related species (Gilly 1978). Further research should focus on adequate soil substrates and treatments, aiming at higher rooting percentages.

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Botanic Garden of Barcelona: past, present and future

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Abstract. *– The city of Barcelona created a botanic garden in 1999 with the aim of displaying the wide range of Mediterranean vegetation in the world. The vegetation is presented geographically according to the five Mediterranean regions of the world. In each region, the plants are grouped according to their ecological affinities. The garden aims to be a centre of reference in Mediterranean plant conservation. It has three main medium and long-term objectives: firstly, the consolidation of the main communities of arboreal and shrub collections from the Mediterranean areas of the world; secondly, the development of a programme of educational activities for the general public and for students; and lastly, to collaborate in the planning and management of the conservation of plant diversity in the region.*

Introduction

The Botanic Garden of Barcelona displays a wide range of flora from the areas of the world with a Mediterranean climate. Mediterranean vegetation is considered one of the richest in species diversity. It has been calculated that less than 5 % of the earth's surface has a Mediterranean climate. On the other hand, the Mediterranean flora includes approximately 25 % of known vascular species (Cowling & al. 1996). At present, it is thought that Mediterranean vegetation is critically threatened, principally by the actions of man. For this reason, the main objective of the Botanic Garden of Barcelona is the conservation of plant diversity in Catalonia and other Mediterranean territories, as well as raising awareness among the citizens with regard to respect for the environment.

Background to the new Botanic Garden of Barcelona

Botanical culture in Catalonia has a long historical tradition. Possibly, the first garden that could be considered a botanic garden was the one created in 1723 in Sant Joan Despí by Jaume Salvador i Pedrol (Pourret 1796), who belonged to the second generation of an extensive family of apothecaries involved in promoting the study of botany in Catalonia during the 18th and 19th centuries. Their collections are now deposited and exhibited in the Natural History Room at the Salvador Museum of the Botanic Institute of Barcelona.

However, the nearest referent for the New Botanic Garden of Barcelona is the Historic Botanic Garden. This garden was created in 1930 (Boix 1989) by Dr Pius Font i Quer in the old stone quarries located in the hollows of Foixarda (Montjuïc Park). In 1940, Dr Antoni de Bolós took over as the director of the garden and increased the collections with vegetation from the Pyrenees and the Balearic Islands. The Historic Botanic Garden has made progress in the great

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task of conserving plant species. The most paradigmatic case is the species *Lysimachia minoriscensis* J.J.Rodr., which completely disappeared from its natural habitat on the island of Minorca (Sáez & Roselló 2001), and could be reintroduced from the live population conserved at the Historic Botanic Garden. At present, the tallest trees in the city can be seen in this garden. Among them are a narrow-leaved ash tree (*Fraxinus angustifolia* Vahl.), a walnut tree from the Caucasus (*Pterocarya fraxinifolia* (Lam.) Spach), and a magnificent camphor tree (*Cinnamomum camphora* (L.) J.Presl.). The spectacular appearance of the stone quarries and its rugged topography, and the large deciduous trees that let a glimpse of the intense summer light through their branches and leaves, make the Historic Botanic Garden a unique place in Barcelona.

The need to build access roads for the Olympic facilities in 1986 seriously affected the stability of the garden. A short time before the 1992 Olympic' Games, closing it down was inevitable. This situation meant that the proposal to fashion a new Botanic Garden in Barcelona that could become the centre of reference for the conservation of the Mediterranean flora in Catalonia was put forward (fig. 1). In any case, the Historic Botanic Garden has not been forgotten, but was opened once again at the end of 2003, in order to continue displaying the collections that Pius Font i Quer had planned at the beginning of the century.

The new Botanic Garden of Barcelona

Within this historical framework, the city of Barcelona created a botanic garden in 1999 with the aim of displaying the wide range of Mediterranean vegetation in the world (fig. 1). The Mediterranean basin, southern Africa, south and southwestern Australia, California, and central Chile are the regions in the world that have a Mediterranean climate. The vegetation is distributed geographically in these five Mediterranean regions. In each region, the plant collections are distributed in an innovative way. The plants are grouped according to their ecological affinities, and therefore, the idea has been to mirror plant landscapes in their natural state. These groups are called "fitoepisodis".

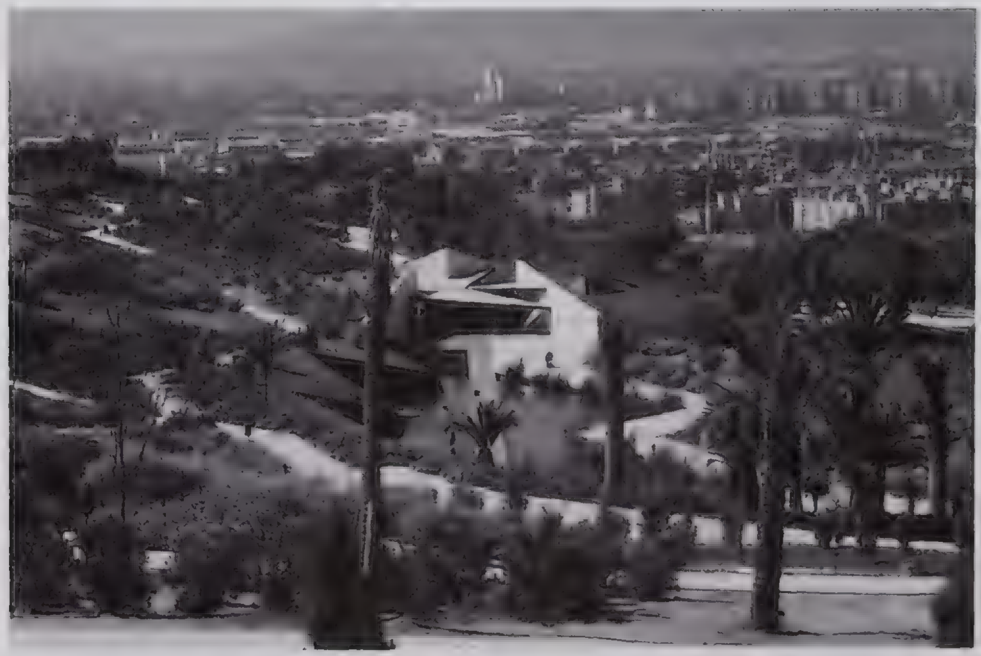

Figure 1. Aerial view of the Botanic Garden of Barcelona.

Visitors are able to discover the observable similarities between vegetation from different zones. In the first place, the arboreal and shrub formations present very similar aspects. In the second place, visitors can observe how Mediterranean plants have adapted and developed, conditioned mainly by the lack of water in summertime. And much more important, visitors can compare the morphological adaptation of different species that come from different regions in the world with a Mediterranean climate.

Three main aspects make this project a model for sustainable gardens in the twenty-first century. In the first place, all the species that are represented are from the Mediterranean area; hence, they have adapted well to the garden's natural climate. In the second place, groups of species with the same environmental requirements allow for a homogeneous adaptation of the representative plant groups to the determining factors of water and soil. And in the third place, the distribution of species in natural landscapes means that a balance of the density of species that is self-regulated by the community itself can be achieved, so that the maintenance and replacement costs of specimens can be reduced to the minimum.

The Botanic Garden of Barcelona now has more than 1,500 species distributed in an irregular manner in the garden. It also has a nursery where about 250 species are grown and then planted in the garden. If the pace of planting is maintained, a collection of about 4,000 species will be reached in 2010.

Main objectives

The Botanic Garden of Barcelona is still a very young garden. It has four principal medium and long-term objectives:

(1) The consolidation of the principal communities of arboreal and shrub collections from the Mediterranean regions. One of the greatest difficulties of the plant collections in the garden is that the exemplified landscapes must contain natural populations of species that are not found in gardening marketplaces. For this reason, most of the plants have to be grown in our own nursery from seed. In order to obtain the seeds, we are making collaborative agreements with other Mediterranean gardens and institutions related to conservation. These agreements make it possible to increase the collections that are included in the garden's Mediterranean phytogeographic areas with the certainty that the wild plant populations that are on display represent a large part of the genetic variability in a plant community.

(2) The development of a programme of educational activities for the general public and for students. The garden is an area for people to spend their leisure time, but it is also an area where one can learn about botany. In this regard, a programme of educational and interpretative activities is being prepared with the aim of opening it up to the city. The most interesting features of the Mediterranean world, which can be discovered in the garden during the four seasons of the year, will be emphasised.

Figure 2. Space earmarked for the display of populations of endemics from Western Mediterranean lands.

(3) To collaborate in the planning and management of the conservation of plant diversity in the region. The Botanic Garden of Barcelona must become consolidated as the centre of plant conservation in Catalonia. In the first place, the Seed Bank of Catalonia has to be created and managed with the help of the regional government. In the second place, it must be responsible for the growth and storing of the species that are threatened in our land. These species are displayed in the garden in order to heighten the visiting public's awareness of the importance of maintaining plant biodiversity in Catalonia. To accomplish this, a variety of rock gardens are being created with the aim of displaying the main endemisms of the Mediterranean basin and to explain the variety of projects that are being developed in the Catalan area (fig. 2). And lastly, the garden is an experimental area with more than 1,500 Mediterranean species that can be used in multidisciplinary research on botany.

(4) To establish research lines with the Botanic Institute of Barcelona that have two main objectives: on the one hand, to maintain a scientific assessment of the garden's collections; and on the other, to develop studies that help towards the further knowledge and resolve the main problems of plant conservation in Catalonia, as well as to encourage collaboration with other countries in the Mediterranean world when undertaking conservation projects. There are many genera shared among the different Mediterranean areas of the world, mainly among regions of the same hemisphere, but also among regions in different hemispheres. This group of vicariant collections allows for the opening up of a wide range of research possibilities in the fields of developmental biology and biogeography of Mediterranean species, which will make it possible for the Botanic Garden of Barcelona to be an emblematic centre of research in the Mediterranean.

Actions planned for the immediate future

The main actions planned to take place at the Botanic Garden of Barcelona by 2007 are:

- To increase and consolidate the garden's plant collections with the most representative species in their natural landscapes. In a later phase, it will be necessary to continue introducing new populations and species in the phyto-geographic areas.
- To equip the garden with the necessary informative signposting to help visitors interpret the displays of Mediterranean plant collections from around the world.
- To determine and to start a scientific research programme with the Botanic Institute of Barcelona, in the field of conservation, which is favourable to the establishment of a conservation plan in the Catalan territory.
- To create the necessary infrastructure and to provide specialised staff to manage the Germ Plasm Bank of Catalonia as an indispensable instrument in plant conservation.
- To create an educational programme of activities for the public in general and for schools with the help of the Association of Friends of the Botanic Garden of Barcelona.
- To increase collaboration with other botanic gardens and other related institutions, such as universities, vocational schools, gardening schools, city councils ..., in order to receive and transmit information at the same time.

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Lectures and workshops as a source of instruction and education on the world of plants

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Abstract. *– The year 2001 marked the beginning of a series of monthly lectures and workshops organized by the Botanical Garden in Ljubljana to the benefit of the general public. Our current activities aim at attracting a yet larger number of visitors to our Garden and instilling into them the need to protect plants and their habitats. The foremost goal of the workshops organized for children was to acquaint them with the plant kingdom, so that particularly town children might develop a better feeling for plants by planting themselves and afterwards taking the pots home.*

Key words: *education, lectures, workshops, botanical gardens, plant biodiversity, habitats.*

Introduction

The Ljubljana Botanical Garden has invested much effort in the programme for schoolchildren presented at the first congress (Bavcon 2000). Our ambitions, however, go beyond this programme, and much is being done to attract also the attention of visitors of other age groups. In this we are handicapped by the fact that the existing Garden's facilities do not allow intensive indoor activies during the autumn and winter. In the year 2001 we renovated the old administrative buildings of the Garden where some rooms are now available for educational purposes. These rooms are not that spacious, and therefore only admitting a small amount of visitors. We started with a series of monthly lectures and workshops organized by the Botanical Garden in Ljubljana for the benefit of the general public. Our current activities aim to attract a yet larger number of visitors to the Garden by e.g. making advertisements in different newspapers, on the radio and television. The Garden regards it as the most important task to promote the knowledge on plants, the need of protecting plants and their habitats, and to popularize horticulture in a general sense.

Methods

Various methods have been introduced for working with visitors and arranging guided tours through the Garden. Visitors are provided with interesting work sheets to fill in during the viewing of the premises in the company of properly educated guides. In the year 2001 a permanent exhibition was opened in the Garden rooms, with as major topic the actual work of the institution. This place is also used for lectures and workshops. The lectures are accompanied by slides, which allows us to comment on plants, the design of some gardens and similar topics. So far we have presented various European gardens and dealt with several related topics. Certain plants associated

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with a garden are given more attention, to catch the attention of the audience with new subjects from this particular domain. Our workshops are based on group working methods where mentors assist children throughout the process. We seek support of the children's parents and invite them to act as mentors. Parents are encouraged to work together with their children and help them to plant their first plants. In this way groups are split up in as small units as possible. To achieve a more lasting effect on the creative imagination of our young visitors, seasonal plants are combined with the evergreen ones so that the pots they take home and continue to take care of always stay green. We normally use plants that are easy to grow, need no special conditions and may also survive outside a protected environment.

If we limit ourselves to the plants of our Garden, people are likely to loose interest and return less frequently. By focusing on different gardens, visitors hear about other parts of the world, admiring the design of attractive gardens, while learning about the diversity of plants resulting in a much more deeper effect.

Our lectures and workshops are advertised in the dailies where daily events are advertised free of charge, in three different garden papers, on the internet, our website and in the city information guide and sometimes on radio stations. Anyone interested in getting information on the events in the Botanical Garden is put on our mailing list.

Results and discussion

Our lectures started with a presentation of the Botanical Garden of Padua, the most ancient garden of its kind, functioning in the same locality ever since its foundation. We proceeded with some English Botanical Gardens: Kew Gardens, Wakehurst Place Gardens, Chelsea Physic Gardens, Birmingham, Edinburgh, Oxford Botanical gardens. A series of lectures dealt with some botanical gardens in Austria: University Botanical Gardens in Vienna, Alpine Garden in Vienna, the Botanical Gardens of Carinthia in Klagenfurt and University Botanical Gardens of Graz. As for Italy, we presented the Trieste Botanical Gardens, the Carsiana Garden and its collection of Karst flora, some other parks and private gardens around villas, and the Carla Fineschi rose garden. One of the lectures was dedicated to botanical gardens of Belgium and the Netherlands: University Botanic Gardens Utrecht, Arboretum Kalmthout, parks of the palaces of Het Loo, Hex and a small garden De Hof van Walenburg. We also took a closer look at the flora of Gran Canaria and the botanical gardens Viera Y Clavijo which we visited during the second Eurogard congress. Afterwards we presented the gardens of southern France, Montpellier, and Nice, with one of the largest gardens 'Bambouseraie Prafrance'. Some of the lecture material was taken from the relevant garden guides and the whole was combined with slides made by the lecturer.

In the year 2002/2003 we started with lectures on cultivars and the corresponding terminology. We presented the Floriade 2002, the horticultural exhibition in the Netherlands. The next topic embraced the plants in terms of the CITES Convention and included a discussion on the trading of the plants protected under CITES (Bolješič 2002). Much enjoyed by our audience was the Eden Project, Cornwall, an optimistic point of the world. The last three lectures were about the secret gardens in Venice, ecological gardening and the woodland gardens in Scotland. At lectures of this type people get the opportunity to learn about new plants, the area where they naturally grow, and garden conditions in which they do well.

Each of the lectures was attended by about 50 people. The lectures proved a great success. The public existed of students, garden lovers, professional gardeners, and people searching for ideas for their own gardens and trying to learn more about plants. The audience at the lectures does not seem to represent a fixed set of people, but those who come at least once, usually repeat their visit to the Botanical Garden. Very popular and well visited events are the free monthly garden tours in the spring time. People show much interest in the plants growing in *ex situ* habitats and have a chance to ask questions about them. Such a tour accompanied by dicussions usually takes more than two hours even though the Garden is actually rather small.

The foremost goal of the workshops organized for children is to acquaint them with the plant kingdom, so that particularly town children might develop a better feeling for plants. Organized for the youngest are simple workshops in which they participate together with their parents. They can plant some plants or listen to a story from the world of plants. School children first take a walk through the Garden to view the plants, then they do their own planting and are allowed to take the pots home with them. We also instruct them how to take care of the plants. Most of the school children like this very much and enjoy participating in such workshops.

Autumn is the pumpkin time in the Botanical Garden. Each year exhibitions of pumpkins are set up. We have a few games for children at that moment and we organise pumpkin workshops. Every child can get a pumpkin to carve his own halloween ghost. Each year more people come; last year we had almost four hundred visitors. Adults like the workshops where they learn about flower arranging, designing greeting cards from dry plants and similar.

Another event that might contribute to the distinction of the Botanical Garden in a more general sense is the issuing of a postal stamp paying a tribute to the 150th anniversary of the birth of botanist Alfonz Paulin (1853-1942) (Marinček 2003). Reproduced in the stamp is his portrait and both of his fundamental works: the Botanical Garden (the then plan) which he headed from 1886 to 1931, and a herbarium of Carniolian plants, *Flora exsiccata Carniolica*, published between 1901 and 1931. This is yet another way of presenting the work of our Garden through its history and of reminding the people of the today's Garden.

The publics interest in such activities increases, however, in spite of that, it is very hard to survive due to the fact that the status of the Garden has not been defined on either the governmental or municipal level. While offering verbal support, the government does not seem inclined to also finance the Garden. This is why so much effort is focused on advertising and popularizing the Garden's work with the general public. We also write in different Slovenian papers specialized for plants, gardens, and others too (The Gardener, The Plant and Gardens, My little world and Scientia). Such public activities were undertaken also in the past but the lack of proper facilities prevented us from inviting people to lectures and similar events.

The aim of our activities is to raise the public awareness about the importance of plant diversity (UNEP 1994), the importance of botanical gardens, to promote conservation and protection of the biodiversity of the plants in the gardens and to share the ideas of (Wyse & Shutherland 2000; Cheney & al. 2000) using botanical gardens as a place where people can learn about plants and to convince the ministries of the important role of the Garden.

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'Children gardens', an international partnership using the Internet

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Abstract. *– Living in towns, children do not have much opportunity to develop a relationship with nature. Since October 2001, children from Brest have been exchanging information about gardens with children from all over the world – Algeria, Spain, Romania, USA, Ireland, Germany Great Britain, Burkina Faso, Italy, and Japan – by means of a website (http://jardinsdenfants.free.fr). Towns in these countries are all twinned with Brest. Each class has created a model of an ideal and imaginary garden, mixing ecological and cultural diversity. Children from eight twinned towns came to Brest in June 2001, to exhibit their models in the City Hall. They also worked together to create a map of the twinned garden that could be constructed in Brest in the future. During the year, this project gave the staff of the Conservatoire Botanique an opportunity to work with environmental educators in Brest, departments of the City, and children from different parts of the world, using nature and cultural diversity as a focus for collective discovery.*

Introduction

From October 2000 to June 2001, the programme 'jardins d'enfants' ('children's gardens') brought together 350 children from the French city of Brest and its ten twinned towns from all over the world. The aim of this programme, which focused primarily on urban green spaces was to raise school children's awareness of biodiversity and urban ecology, as well as to widen their cultural outlook and stimulate their imagination, their observational abilities and aesthetic awareness. The Internet proved the best available tool for communication between continents, to make acquaintances and to exchange views. During the course of the school year, the children constructed a model for their ideal garden, and then got together in Brest in June 2001, to set up the plan of the twinned garden.

The roots of the project

In the previous year, a similar venture involving a partnership between the Conservatoire Botanique National de Brest, a plastic artist (Gaëlle Kéroullé), the environmental staff from wildlife societies, and the City Environmental Services, had been undertaken with two primary schools from Brest. This first effort was so successful that Gaëlle Kéroullé decided to develop the scheme on an international level. The aim of the scheme was to involve schoolchildren from Brest and from another ten towns twinned with Brest.

Supported by the City Council Member responsible for twinning, the education team set up an association entitled 'Jardins d'enfants'. This enabled us to obtain funding specifically for the

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programme and to employ Gaëlle Kéroullé as coordinator for a full year. Once every month or so, the Brest education team met to assess the progress of the programme.

Children living in towns often have limited access to and limited knowledge of the environment. This was why we focused on urban green spaces as the framework for this initiative, since all towns have their own open spaces, which often offer the only bit of nature in the neighbourhood where children can play and express themselves freely. The idea was to show the children that this place they regularly go to, can prove a stimulating place to discover, once conditions are provided for doing so. The scheme started by asking the children to illustrate their best souvenir of nature. This enabled their teachers and educators to get an idea of the way the children perceived the natural world as compared to their urban environment, as well as to assess their achievements by the end of the programme.

In Brest as well as in the ten twinned towns, the principle was to have each one of the classes concerned work throughout the year, on one particular urban green space near their school, studying it successively from different angles, under the supervision of educators with different specialities. For instance, the two classes from Brest, tackled the following themes:

- the creation and maintenance of an urban green space, with the gardening staff of the city;
- wildlife, with an educator from a local wildlife trust;
- plant biology, with an educator from a scientific society;
- native and exotic trees, and plant threatened by extinction with an educator from the Conservatoire Botanique;
- energy sources (photosynthesis, wind and hydraulic power…) with a science teacher;
- the artistic and amusement approach to green spaces with an artist.

In the course of these sessions, the children were able to grasp the concept of urban green areas, and their perception of these was broadened and diversified. Around the middle of the year, it was possible to ask them to prepare a plan for a recreation and play ground.

Children join in the game

Having grasped various concepts relevant to urban green spaces, the children could then draw up the maps for their ideal garden. The conception of these gardens by them, and most importantly, for them, drew on their unlimited imagination. In this way, little by little, appeared on paper urban spaces under various names: 'prehistoric corner', 'the color garden', 'the tentacle garden', play grounds such as 'the bouncy hills', 'the orientation garden' or 'the live plant labyrinth' and also some poetical spaces such as 'the beautiful grove', 'water reflections' and 'the peaceful garden'.

In order to conceive these gardens, children searched for information by themselves, about the building materials, the objects, the plants and the animals which they wished to have in their spaces. They could also ask their various educators for more information, via the Internet. For instance, for Audrey who wanted a prehistoric corner with owls, The Conservatoire Botanique provided her with a list of plants, which have existed for long on the earth such as Ferns, Conifers, Magnolias, and amazing plants such as Gunneras. The wildlife educator gave her hints as to how to attract owls and have them nesting in her garden. From these suggestions, the children picked out those species, which they preferred, and which were also compatible for one another.

When their garden spaces were clearly defined, the children joined together to assemble these in a common plan and thus create the class garden (fig. 1). With the help of the artist and their teacher, the pupils built a small scale model of their ideal garden. The classes of the ten twinned towns worked along the same lines as the two Brest forms.

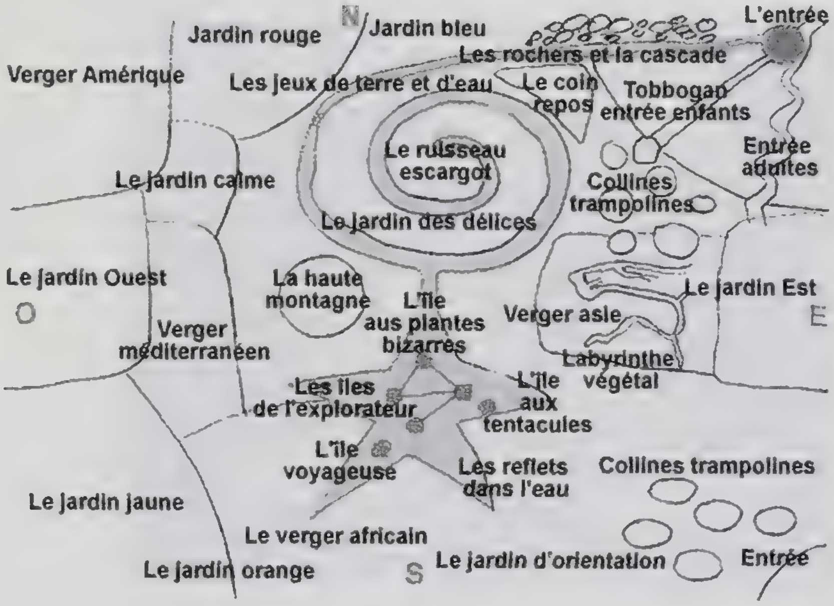

Figure 1.
Children from Brest:
map of their ideal garden.

Internet: communication and exchanges

The classes were told about the "children's gardens" scheme in October 2000. They sent their applications to enter the scheme and presented their class and their town. This information was put on the 'children's gardens' website (http://jardinsdenfants.free.fr) and the text was translated into French and English.

In November, a first 'cyber-meeting' took place to enable the children to exchange ideas and this proved a success. Every two months or so a News-letter entitled "Turdus News" was sent to all partners involved in the programme, recounting what was being done and how things were progressing in the eleven towns. A second 'cyber-meeting' took place in May so as to prepare the June meeting in Brest between the twinned towns.

The Internet proved the ideal tool for the children to exchange information and, as the web site developed, to follow the progress of the project, and to maintain the interest of the participants.

The Brest meetings

The meetings that took place in Brest between the child ambassadors involved all the other people concerned as well. Although these meetings were an opportunity for amusement, the children had an important target before the end of their sojourn: to design the plan of the twinning garden which may one day be set up in Brest.

A whole day was needed to achieve this result. In the morning, the children from each twinned town chose the elements they preferred from the different plans drawn up by their comrades from the other towns, and which they wished to retain in their working draft. In the afternoon, each one of the groups presented their proposal and a wide debate was organised in order to select the elements to draw up the final model. This step was particularly arduous since the discussion had to be simultaneously translated into five different languages so that everyone could understand what was going on and express their own view. But we were not building a tower of Babel and we did manage to accomplish this step and design the final model for the "twinned garden". The next day, the children colored the map of the twinned garden and displayed their model in the town hall of Brest. At the end of the day, the city councillors opened the exhibition presenting the different twinned towns and the models built by the children (fig. 2). There, the children officially offered the plan for the twinned garden to the City Councillor responsible for twinning. The wish that everyone expressed was that one day the map may serve as the basis for the creation of a real garden in Brest.

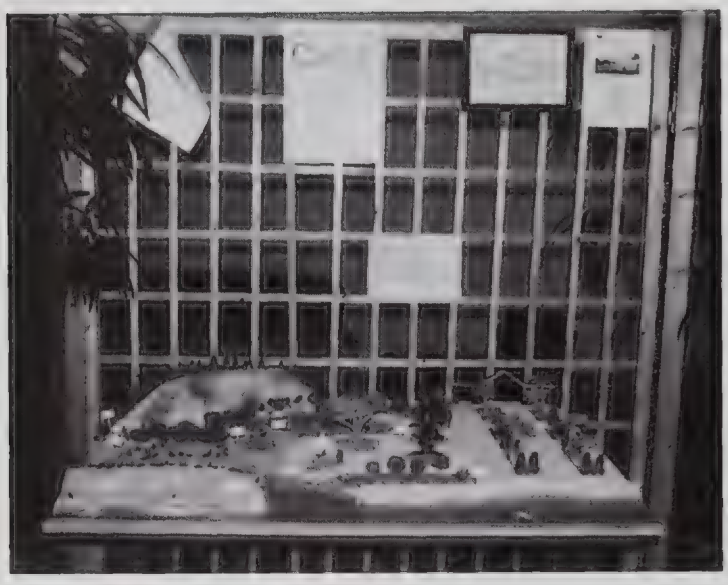

Figure 2. The model from the school of Cadiz.

But the children were not in Brest only to work! This was above all a wonderful opportunity for children, aged from 10 to 15, from strikingly different cultural backgrounds, to meet, play together and discover our town and our way of life. Amongst other lively events in the course of that week, they will remember:

- a big discovery trail in a wooded park;
- the unveiling of the models and the official opening at the Town Hall;
- the meeting with the City Councillors;
- the evening party, in a youth hostel;
- the twinned garden celebration party itself.

The twinned garden party

On the eve of the children's departure, a big party was organised in a public park close to the Town Hall, in order to open this twinning project to the whole population of Brest. This pleasant afternoon ended with the child ambassadors planting a young oak to celebrate the meeting and to consolidate the twinned garden project. Oak acorns were offered to the authorities of the twinned towns so they could plant them in their respective countries. These acorns were to be the living symbol of the generous sharing that marked this scheme, and the perennity of the relations it established between children of different cultures.

Whether for the Children from Denver or those from Saponé, this twinning focusing on environment education enabled children and educators to exchange and develop ideas about and deepen understanding of nature, and of ways to enjoy and acquire these notions through an approach, which was both scientific and imaginative. The Internet made this possible and proved fruitful for all. This experience also showed that it was possible to bring children together from different origins, cultures and mother tongues and to have them work on a common project, stimulating a desire both to discover and to share with others. The games, the exchanges, and the debates raised by this meeting in Brest will certainly leave their marks, as the smiles, the laughter and the bright eyes clearly indicated.

Today, the Internet site and the oak planted by these emissaries in the heart of Brest, bear witness to the wonderful scientific and cultural success the meeting produced. Let us hope that this simple and generous action may inspire new initiatives and nurse the desire to bring people together and discover more about each other and the natural world, which surrounds them.

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Is there a role for horticultural research in Botanic Gardens?

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Abstract. *– Research in botanic gardens has traditionally been restricted to taxonomic and systematic work. However, the comprehensive range of plants cultivated in botanic gardens and the quality of documentation usually attached to them means that the collections are an excellent resource for some types of horticultural work. Many types of horticultural research, especially in those fields devoted to commercial crops, are better left to specialised research stations. However, research that is compatible with, and that supports, traditional types of botanic garden work are highly appropriate for botanic gardens. This paper considers some examples including phenological observation, cultivation trials, flower initiation, seed technology, conservation, growing media and horticulture-led taxonomy.*

Introduction

It is well understood in the business world that maximisation of resources, in other words of deriving as much benefit as possible from one's resources, is a key factor in delivering efficiency and profitability. This is especially true of expensive resources that, if not used to their full potential, are simply a waste of money. Living collections in botanic gardens are expensive resources, having cost a lot to amass and maintain and so the same type of thinking might be applicable. Traditional uses of the collection are based predominantly around systematic and taxonomic research, but could it not be argued that restricting their use to this type of work only is a case of not maximising resources?

This paper considers the case for expanding the traditional research role of live plant collections in botanic gardens to include carefully selected horticultural research.

Traditional research

Traditional forms of research in botanic gardens include taxonomy, systematics, evolution and phytogeography which contribute to end uses such as floras, monographs, ecology and population dynamics. Herbaria, library and archive collections are used to support this research, but so too are live plant collections. Live plants provide DNA for molecular studies, roots tips and other parts for chromosome and cytological research and they are held over time so that plant development and orientation can be observed. They also provide good taxonomic material where herbarium specimens are poor, for instance with succulents and orchids. There are many good

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reasons, then, why living plants are cultivated in botanic gardens to support these examples of 'traditional' research.

Live plant collections in botanic gardens

Live plant collections in botanic gardens are generally large, diverse and well documented. They are frequently comprehensive in one or more categories such as native plants, plants from other geographic areas, or in certain families or genera. Other examples of specialisation include plants from particular habitats or growth forms, e.g. alpines and aquatics or bulbs and herbs. Such concentrations of plants by geography, taxonomic status or ecological niche, allied with good records, make these collections valuable research tools and seldom could any research worker access such a diversity of material on a single field trip, much though field trips are vitally important. These collections, then, represent an expensive and hugely valuable reseach resource from which botanic gardens should surely aim to extract the fullest possible use.

An illustration of the diversity of just one collection, that from the Royal Botanic Garden Edinburgh (RBGE), is shown in table 1.

Table 1. Facts about the collection of the Royal Botanic Garden Edinburgh.

Species	15,420
Taxa	20,550
Accessions	41,561
Plants	66,444
IUCN status taxa	1,327
CITES (I & II) taxa	1,375
Family representation - 5 largest families in collection	Ericaceae, Rosaceae, Compositae, Orchidaceae, Umbelliferae
Geographic representation - 5 largest collections by country	China, USA, Nepal, Chile, UK

Prerequisites for use

Just as living collections require to be of a certain standard to be of use for 'traditional' botanic garden research, so they also must have certain prerequisites before they can be useful for horticultural research. Luckily, however, there is a good deal of overlap between the standards required for these two types of work that further strengthens the argument that horticultural research is valid in botanic gardens.

These prerequisites basically include a high percentage of wild origin material, high level of verification, a consistent and high standard of documentation and a comprehensive representation of material, be that at genus or family level, by country of origin or by habitat.

Wild origin material is important because it means that a plant's exact locality and background are known. If the material is of 'garden' origin, i.e. from an unknown source such as a commercial nursery or other botanic garden the material might be of hybrid origin and, even if not, its exact origin is probably unknown. Many species have very wide distributions and contain, accordingly, large variations in morphology. Without knowing the exact origin of a plant it is very difficult to draw firm conclusions about any aspect of its taxonomic or systematic status.

Verification is important because it confirms the exact name of the plant. Without the confidence of being sure that the correct name is attached to a specimen it is difficult to compare the specimen to other species or carry out any investigation into the plant because of the uncertainty of the status of the material being studied. Good documentation is one of the main factors that separate botanic garden collections from other plant collections and is absolutely fundamental to any potential research. The records attaching to a plant describe the plant's background including original collection details such as altitude and habitat and also subsequent history such as propagation method, transplanting and landscape performance.

Frequently, a botanic garden collection is useful for research because it is comprehensive in some way. While research workers often work on individual species, others are looking for a 'good range' of material from within certain categories – this could mean many species within a genus, or many species from a particular geographic area, habitat type or other plant grouping (such as conifers, bulbs or alpines). It is just the sort of combined prerequisites listed above that are frequently, and also uniquely, found in botanic garden collections. Table 2 shows percentage wild origin and verification at RBGE. Largest families and geographical areas are shown in table 1.

Table 2. Wild origin and verification at the Royal Botanic Garden Edinburgh.

	%	Target	Notes
Wild origin	54.8	Increase by 1% per year	New material ranges from 79-92% over last 10 years
Verification	24.2	Increase by 2% per year	Targeted verification process now in place (Cubey & Gardner 2003)

Inappropriate horticultural research

Before starting to describe suggestions for suitable horticultural research in botanic gardens it is important to note that for most botanic gardens there are many types of horticultural research that are completely inappropriate. These basically include anything that others are better suited to or equipped for. Examples include crop trials, pesticide development, the development of new cultivars and disease resistance. These types of research, which are generally related to commercial or production horticulture, are far better left to specialist horticulture research stations that are specifically equipped for this type of work.

In listing potential types of research it is not particularly useful to single out 'horticultural' research as opposed to what has been described above as 'traditional' botanic garden research such as taxonomy – in the end it is all plant-based research and any new knowledge that can be derived from botanic garden collections should be applauded irrespective of the label attached to it. In this paper the phrase horticultural research has been singled out as it is the main point of the paper. However, in the end it would be better if no distinction was made between the two-they are all simply plant research that takes place in botanic gardens.

So, what might be regarded as appropriate research? It is generally research that can be grouped under the following headings: research that supports 'traditional' botanic garden research as described above, research that is not being done by others and research that makes best use of the collection and of the landscape.

Phenological observation

This work may not be regarded as research by some but it certainly needs a careful and methodical approach to observation and recording and, once sufficient data has been kept for some time research opportunities in fields such as climate change and flower initiation become possible. Botanic gardens and their collections are particularly suitable for this type of work because of their comprehensive collections, tradition of careful record keeping, general stability and longevity (records need to be kept over a long period of time to have any value) and because many gardens also keep meteorological records. Following a lapse of many years phenological data is again being collated at RBGE. Various approaches are being adopted and to start with 'first flowering date' information is being recorded for 100 selected individual plants with checks taking place every day, thanks to a particularly dedicated volunteer. More recently a selection of six bulbs (asexually propagated) have been planted closely together in six different microclimates within RBGE (e.g. south facing and north facing bank) and flowering dates will be recorded to, hopefully, show up microclimate changes. Finally, it is hoped that a 'what's flowering this week' record will be initiated by a group of volunteers. Rather than recording the first flowering date it will simply record which plants are flowering in each week of the year.

Cultivation trials

Cultivation trials are of course, not new to botanic gardens and have been going on for as long as botanic gardens have been in existence. What could be new is a more methodical approach to such trials and, perhaps even more importantly, the recoding of the information gained from the trial. This type of horticultural research is entirely appropriate to botanic gardens as it supports the so-called 'traditional' type of research described earlier. By finding ways to grow 'difficult' plants there can be obvious benefits both to taxonomic work and to public education and display - if important plants cannot be cultivated then live plants cannot be of any benefit to taxonomic research. Likewise badly grown plants that are only just surviving rather than flourishing are of little value to public education or display. Examples at RBGE include the cultivation of 'difficult' cerrado vegetation species due to soil chemistry complications and the (shortly to be undertaken) cultivation trials of the critically rare Scottish native *Calamagrostis scotica* (Scottish small-reed) which is difficult to grow *ex situ* due to the fluctuating soil hydrology found in the wild. A suitable outlet for this type of work does not currently exist and this is the reason why RBGE has launched the new journal *Sibbaldia* (Rae 2003) which is an occasional series of horticultural notes. While only one issue has been published to date it is hoped that the journal will expand to include more articles and be published at greater frequencies than the current rate of one issue per year.

Flower initiation

Again, this links directly to taxonomic research, especially to so-called alpha taxonomy. Seedlings collected in the field that subsequently fail to flower can cause frustration and delays in morphological description. At RBGE, for instance, the lack of flowering in a number of potentially 'new' Section *Vireya* species of *Rhododendron* held up their description for a number of years. Seedlings had been collected from near to what was possibly thought to be an undescribed species during the non-flowering season. Herbarium specimens were of no help and so the live plant introductions were potentially very useful, except for the fact that they didn't seem to flower. Some rather simplistic, non statistically valid (due to shortages in specimen numbers) trials were undertaken using day length and diurnal temperature changes. Even though these trials were unsuccessful the illustration does serve, however, to show that this sort of work in botanic gardens is valuable and links horticultural and botanic research perfectly.

Seed technology

Botanic gardens are more interested in species variation rather than clonal selection and therefore generally collect plants as seed rather than as cuttings (unless plants are not in seed at the time of collection). Thousands of species enter botanic gardens as seeds each year and, while there are frequently some attempts to germinate 'difficult' species, a methodical approach to seed germination seems to have been absent in most botanic gardens. Small seed germination investigation units would, in the author's opinion, have been much more valuable than the plethora of micropropagation units that sprang up in botanic gardens in the 1980s and 1990s, despite the valuable work done in such units such as at RBG Kew and at Jardín Botánico "Viera y Clavijo" in Gran Canaria. At RBGE the International Conifer Conservation Programme has had a notable successes with the hitherto difficult to germinate conifer species *Podocarpus nubigenus* and is currently working on *Acmopyle pancheri* and *Prumnopitys andinus*. Their research on seed germination was carried out in conjunction with the Forestry Commissions Research Station, Alice Holt.

Conservation

Integrated conservation calls on a number of approaches to conserve species and habitats. It is the combination of different approaches, using the skills of different sectors that can often bring real conservation benefits, rather than the efforts of an individual action. *In situ* and *ex situ* conservation, education, habitat management, population genetics and other disciplines can combine very effectively in these integrated approaches. Horticultural research comes into play again though not just as *ex situ* work but also in re-introduction programmes and *in situ* conservation. Fundamental issues such as germination, cultivation and post re-introduction management require horticultural techniques which, when not working well, can call for trials and research. Put simply, we need to be able to cultivate threatened species well if we want to be able to re-introduce them and horticultural skills and investigation have an important part to play.

Botanic gardens have a unique mix of staff: people who can research, grow and explain plants and horticulturists can now play their part in conservation by taking a more methodical approach to the cultivation and re-introduction of threatened species. The research work of Dr Kim Tripp, President of Horticulture at New York Botanic Garden is worth quoting in this context. Dr Tripp has been using the extensive and well documented collections of *ex situ* grown conifers gathered by the International Conifer Conservation programme mentioned above. The principle aim of her research has been to investigate the use of cone induction techniques as a method of producing viable seed from selected threatened conifer species and its application in conservation programmes. A secondary aim has been to investiagate the reproductive biology of conifers such as *Fitzroya cupressoides*.

Growing media

Compost trials are one of the most obvious cultural factors that can be manipulated by horticulturists. Both the texture, structure and physical composition can be altered, along with the chemical composition. A lot of work has been done in the past but now a lot of time is being devoted to peat alternatives. At RBGE for instance, following extensive trials, the entire *Rhododendron* Section *Vireya* collection has been cultivated in a non peat medium for a number of years now.

Horticulture-led taxonomy

With so many wild species still awaiting description and identification it is obvious that the main thrust for taxonomists and systematic research should be on plants growing in the wild rather than on cultivated plants. The latter do, however, require some special skills to identify and classify, have a taxonomic code of their own and have obvious end users in the form of amateur gardeners and the nursery trade. Several botanic gardens, including RBGE, have an interest in horticultural taxonomy and the staff member(s) concerned often play a very useful role in helping to verify and curate the living collections. Their research work is often devoted to garden floras (for instance the European Garden Flora), the taxonomic description of garden plants and cultivars and the reclassification of important groups of garden worthy plants such as *Rhododendron* and *Viburnum*. The living collections are a good source of material for such work in general but a particularly good specific example of the value of this type of work and of the use of live plant collections has taken place in RBGE over the last few years. The identity and taxonomy of several highly garden-worthy *Meconopsis* species and cultivars had become very confused over the last twenty years or more. In an attempt to sort out the various problems RBGE agreed to act as a central holding ground for numerous clones of the various problematic *Meconopsis* that were sent in by nurseries, botanic gardens and enthusiastic amateurs. These plants were grown in close proximity and became the focus of study for a group of *Meconopsis* enthusiasts. After several years of careful observation and discussion the taxonomy and identity of the species and cultivars under investigation was eventually cleared up and a publication explaining the results was published (Stevens & Brickell 2001). Having successfully completed that group of *Meconopsis*, other taxonomically confused groups within the genus are now being studied. This case study again illustrates the value of live plants in botanic gardens in supporting horticultural research.

Conclusion

Horticultural research is legitimate in botanic garden but it should be compatible with the mission of the garden. There are some types of horticultural research that are better done in other institutions. Generally, research that supports the core research aims of the garden are the most appropriate. Suitable types of research can generally be described as cultivation techniques - germination, cultivation and growing media, as these link directly to taxonomic and cultivation research. Even 'observation' and non-statistical research is valuable but we must make better efforts to record our knowledge.

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Phenology of tropical and subtropical plants in greenhouses in the National Botanic Garden of Belgium

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Abstract. – *Since 1993, the flowering and fruiting behaviour of hundreds of tropical and subtropical plants grown in the greenhouses of the National Botanic Garden of Belgium are studied. Each taxon is observed every two weeks during at least five years; its flowering and fruiting periods are then expressed in number of weeks (from 1 to 52). Fourteen types of flowering or fruiting periods are distinguished, based on duration and frequency. Some periods are very short, others are spread over several seasons, others occur two to several times a year. A calendar of flowering is established which is useful for horticulturists, collection managers, gardeners and for the general public visiting the greenhouses.*

Key words: *flowering behaviour, fruiting behaviour, calendar of flowering phanerogams*

Abbreviations: *FL, flowering; FR, fruiting*

1 Introduction

Plants grown in the tropical and subtropical greenhouses of the National Botanic Garden are studied with regard to flowering and fruiting behaviour. The observations started in 1993 and are still going on. Several hundreds of different taxa belonging to more than hundred families have been studied until now. A series of first results has been published (Billiet 1999). In literature, most of phenological research has been carried out on natural populations in the wild. Very few studies have been conducted on plants grown in greenhouses (Hamilton 1990).

The vast collections of plants grown in the greenhouses of the National Botanic Garden give a good opportunity to study their floral and fruiting behaviour. Phenological data are important for programming the sampling of seeds needed for the seed bank and the annual Index Seminum. They are useful for biologists, gardeners and can be of interest to the general public. Moreover, making phenological observations enables the scientific manager of the collections to verify the identity of the living plants at the right moment.

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Table 1. Classification of flowering and fruiting stages.
* Stage 3 indicates that open flowers are present together with flower buds and withered flowers; this stage may be used for a single open flower.
** Stage **3** (bold underlined) is used when a spectacular or mass flowering is observed.

Stages of flowering		Stages of fruiting	
1	flower buds	1	initiation of fruits
2	buds + some open flowers	2	young green fruits
3	buds + open flowers + withered fl. *	3	green + mature fruits
3	peak flowering **		
4	open flowers + withered flowers	4	all fruits mature, possibly fallen
5	all flowers withered	5	all fruits fallen
7	1 + 5	7	1 + 3 (2 generations of fruits)
		8	1 + 4 or 2 + 4
9	flowers dried, aborted, ...	9	fruits dried, aborted, ...
.	no flowering	.	no fruiting

Alectryon subcinereus **(Sapindaceae)**

```
        J   F   M   A    M   J    J   A   S    O   N   D
                 1         2         3         4         5
        1234567890123456789012345678901234567890123456789012
1993 FL                          1 1 1 2  3  4 5 5 5
1993 FR 11112222333   4  4  5
1994 FL                          1 2 3 3 4 4 4 4 4 5 7 4 5
1994 FR                                  1 1 1 1 2 2 2 2 2 2
1995 FL                                1 3 3 3 4 4 5
1995 FR  2 3 2 2 3 3 4 4 4 4 4 5 5 5           1 1 1 1 1 1 2
1996 FL                            1 1 1 1 2 3 3 3 4 4 5 5 5
1996 FR  1 1 2 2 2 2 2 2 2 2 3 4 4 4 5 5
1997 FL  5                                     1 1 1 1 1 1 1
1998 FL 1 1 1 1   1 9      1 1
```

Euclinia longiflora **(Rubiaceae)**

```
        J   F   M   A    M   J    J   A   S    O   N   D
                 1         2         3         4         5
        1234567890123456789012345678901234567890123456789012
1996 FL                                                1 3 5
1997 FL                                              1 1 3 4
1998 FL 5                                                 3
1999 FL  5                                           1 2 4
2000 FL                                               2 3 4
2002 FL                                              2 3 3 4
```

Table 2. Global tables of four species studied showing annual evolution of flowering (FL) and fruiting (FR) stages.

2 Methods

Plants were examined every two weeks during at least five years. At each visit the stage of flowering and fruiting is noted according to a nine step classification (table 1). After five years of observations of a taxon, a global table (table 2) is obtained which gives for every week and every year the stages of flowering and fruiting. All the original results have been computerized in the databank LIVCOL which is in use at the National Botanic Garden of Belgium for the management of the living collections (www.br.fgov.be/RESEARCH/PROJECTS/livingplantcollections.html#database).

These global tables allow to specify the extension of the flowering and fruiting periods for each species. More refined informations may result from the interpretation of these data such as the time necessary to develop from young flower bud to full anthesis, from fruit initiation to mature fruit etc. These kinds of results are not discussed here and may be interpreted later on. Here some possible output on flowering and fruiting periods is illustrated. These periods are classified in different types of behaviour and finally a calendar of maximum flowering is presented.

3 Flowering periods

The periods of flowering correspond to the time during which at least some flowers are open, thus including stages 2, 3, 3̲ and 4 (2 = buds + some open flowers, 3 = buds + open flowers + withered flowers, 3̲ = peak flowering, 4 = open flowers + withered flowers).

Heliconia humilis **(Heliconiaceae)**

```
          J   F   M   A    M   J    J   A   S    O   N   D
                   1         2         3         4         5
          1234567890123456789012345678901234567890123456789012
1993 FL                                                 1
1993 FR             4 4 4 4
1994 FL       5 5 5
1994 FR       3 3 3 3 555 5 5
1995 FL             3 5 1 3 3 5                   1 2 3 3 3 4
1995 FR             3 4 5   4 4                         3 4 4
1996 FL   5 7 2 3 3 3 3 4 5                         1 1 2 3 3
1996 FR   4 5 4 4 4 4 5 4 4 4 4 5
1997 FL   4 3 4 5 7 3 3 5   7 3 3                         4 4
1997 FR       4 4 8 4 4 4 5       4 5 4 4 5
1998 FL   5       3 3 3  3 5
1998 FR    4 4            4 4 4 4 4
```

Mackaya bella **(Acanthaceae)**

```
          J   F   M   A    M   J    J   A   S    O   N   D
                   1         2         3         4         5
          1234567890123456789012345678901234567890123456789012
1993 FL   111222222223 3 33 4 5
1994 FL   1 1 1 1 1 2 3 3 3 4 4
1994 FR                     .   1
1995 FL   1 1 1 1 2 2 2 3 3 3 4 4 5 5                   1
1995 FR                       1 1
1996 FL         1 1 1 1 2 3 3 3 4 5
1997 FL                     3 3 3 4 4 5 4 5
1998 FL                   1 2 3 4 9
1999 FL                 2 3 3 3 4 5
```

Evolution during several years (weeks 1 to 52). Stages of flowering and fruiting as in table 1.

Table 3. Stages with open flowers of *Alectryon subcinereus*.
Evolution during several years (weeks 1 to 52).
Stages of flowering as in table 1; – absence FL; * flowers not open

weeks	26	28	30	32	34	36	38	40	42	44	46
1993	*	*	*	2	2	3	4	*	*	*	–
1994	*	2	3	3	4	4	4	4	4	*	*
1995	–	–	–	*	3	**3**	3	4	4	*	–
1996	–	*	*	*	*	2	3	3	3	4	4
1999	3	3	3	*	–	–	–	–	–	–	–

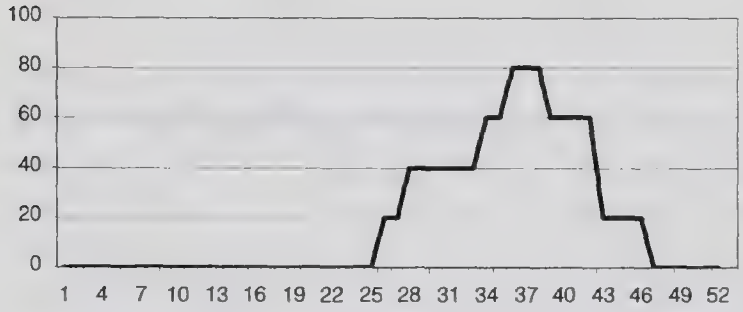
Table 4. Some parameters showing flowering behavior of *Alectryon subcinereus*.
A : annual start and end of flowering period (stages 2, 3, **3** and 4) in number of weeks, B : duration in weeks of flowering period,
C : annual start and end of maximum flowering period (stages 3 and **3**) in number of weeks,
D : duration in weeks of maximum flowering period.

	A	B	C	D
1993	32-38	6 weeks	36	2 weeks
1994	28-42	14 weeks	30-32	4 weeks
1995	34-42	8 weeks	34-38	6 weeks
1996	36-46	10 weeks	38-42	6 weeks
1999	26-30	4 weeks	26-30	4 weeks
mean value		8.5 weeks		4.4 weeks
extreme values of duration of FL	26-46	20 weeks		
extreme values of duration of maximum FL			26-42	16 weeks

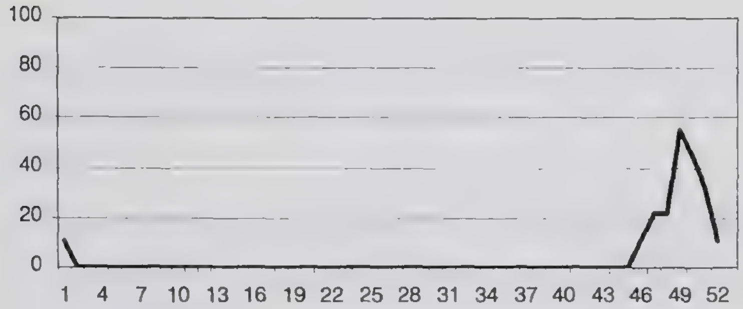
Table 5. Calculation of mean values of flowering (in %) of *Alectryon subcinereus*.
– absence of FL.

weeks	26	28	30	32	34	36	38	40	42	44	46
1993	–	–	–	1	1	1	1	–	–	–	–
1994	–	1	1	1	1	1	1	1	1	–	–
1995	–	–	–	–	1	1	1	1	1	–	–
1996	–	–	–	–	–	1	1	1	1	1	1
1999	1	1	1	–	–	–	–	–	–	–	–
total	1	2	2	2	3	4	4	3	3	1	1
%	20	40	40	40	60	80	80	60	60	20	20

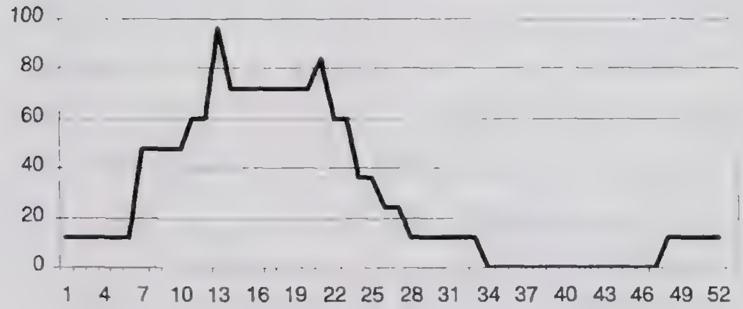
Figure 1.
Flowering behaviour
of four examples of species studied.



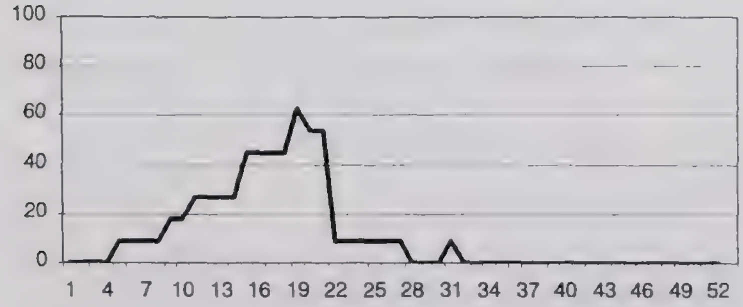
Alectryon subcinereus



Euclinia longiflora



Heliconia humilis



Mackaya bella

For example, the global table of *Alectryon subcinereus* can be simplified to show only the stages with open flowers (table 3). The total period showing open flowers runs from week 26 to week 46. From this simplified table several flowering parameters can be calculated (table 4). For *Alectryon subcinereus*, the duration of the flowering period (column B) for individual years varies between 4 and 14 weeks; the mean value is 8.5 weeks. The duration of the maximum of flowering (column D) varies between 2 and 6 weeks; the mean value of the maximum flowering period is 4.4 weeks. The extreme duration of flowering covers 20 weeks and that of the maximum flowering covers 16 weeks. The extreme values of beginning of flowering (column A) can show a difference of 10 weeks; for the start of the maximum flowering (column C) this difference is 12 weeks.

A diagram can be drawn when the stages showing open flowers of table 3 are expressed in percentage. In our example of *Alectryon subcinereus*, all the figures of table 3 are transformed in "1" (table 5). The sum is then expressed in percentage and can be figured in a graph of the floral behaviour. Figure 1 shows the floral pattern of four examples of species studied. These graphs can easily be compared and they illustrate different types of flowering behaviour (see further).

4 Fruiting periods

The fruiting behaviour can be analyzed in the same manner. The periods of fruiting correspond to the time during which mature fruits are present (fruiting stages 3 and 4).

For example, the global table of *Alectryon subcinereus* (table 2*)* can be simplified as follows to show the stages with mature fruits (3 = green + mature fruits, 4 = all fruits mature, possibly fallen) (table 6). The total period showing mature fruits runs from week 4 to week 28.

In a similar way as for the flowering data one can calculate some fruiting parameters (table 7) and represent the fruiting behaviour in graphs (fig. 2).

Table 6. Stages with mature fruits of *Alectryon subcinereus* during several years.
Stages of fruiting as in table 1. –, absence of fruiting; *, fruits not mature.

weeks	4	10	12	14	16	18	20	22	24	26	28
1993	*	3	3	3	4	4	*	–	–	–	–
1994	–	–	–	–	–	–	–	–	–	–	–
1995	3	3	3	4	4	4	4	4	*	*	*
1996	*	*	*	*	*	*	*	3	4	4	4

Table 7. Some parameters showing fruiting behaviour of *Alectryon subcinereus*.
A : annual start and end of fruiting period (stages 3 and 4) in week numbers, B : duration in weeks of fruiting period, C : annual start and end of fruit dispersal period (stage 4) in number of weeks, D : duration in weeks of fruit dispersal period.

	A	B	C	D
1993	10-18	8 weeks	16-18	2 weeks
1995	4-22	18 weeks	14-22	8 weeks
1996	22-28	6 weeks	24-28	4 weeks
mean value		10 weeks		5 weeks
extreme values of duration of fruiting	4-28	24 weeks		
extreme values of duration of fruit dispersal			14-28	14 weeks

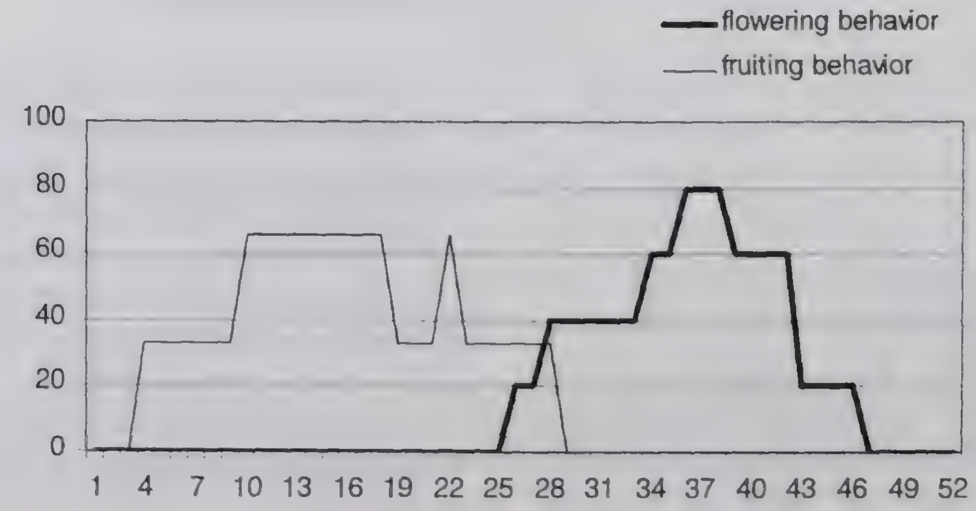


Figure 2. Fruiting behaviour of Alectryon subcinereus, *compared with flowering behaviour.*

5 Types of flowering and fruiting behaviour

After having analyzed several hundreds of taxa, the existence of different types of behaviour became evident. Fourteen types of behaviour are distinguished based on duration and frequency of flowering or fruiting periods (table 8).

The flowering periods have been extracted from the global tables showing the annual evolution of flowering of each taxon studied. The periods of flowering and fruiting often correspond to several plants. One taxon may be represented by several accessions and plants belonging to one accession may be located in different greenhouses. If the periods of different plants do not overlap, all the values (except the very abnormal ones) are taken into account; this results in a broader period of flowering or fruiting for the whole of the accessions of one taxon.

Five examples of different flowering types are presented in table 9.

The complete updated results on periods and types of flowering and fruiting made from 1993 onwards on several hundreds of species is to be found on:
www.br.fgov.be/RESEARCH/DATABASES/phenology.html
A small sample from this long list is given in Appendix 1.

An enumeration of all the species studied, classified according to the fourteen types of flowering behaviour and the fourteen types of fruiting behaviour is to be found in Appendices 2 and 3.

Table 8. Types of flowering and fruiting behaviour.

types of flowering or fruiting	season of flowering/fruiting	period (number of weeks) during which flowering or fruiting occurs
1	spring	13-25
2	summer	26-38
3	autumn	39-51
4	winter	52-12
1-2	spring-summer	13-38
2-3	summer-autumn	26-51
3-4	autumn-winter	39-12
4-1	winter-spring	52-25
1-2-3	spring-summer-autumn	13-51
2-3-4	summer-autumn-winter	26-12
3-4-1	autumn-winter-spring	39-25
4-1-2	winter-spring-summer	52-38
1-2-3-4	continuous flowering/fruiting	
5	two or more flowering/fruiting periods in one year	

Table 9. Extreme periods of flowering observed during at least five years, flowering season and type of flowering.
Five examples of taxa studied.

Taxon	Extreme periods of flowering (number of weeks)	Flowering season	Type of flowering
Alectryon subcinereus	26-46	summer-autumn	2-3
Euclinia longiflora	47-52	autumn	3
Heliconia humilis	45-25	autumn-winter-spring	3-4-1
Eichhornia crassipes	23-26, 42-48	spring and autumn	5
Mackaya bella	9-27	winter-spring	4-1

Some species have a very short period of flowering or fruiting; they are listed in Appendices 4 and 5. When comparing the flowering/fruiting periods of these taxa one year after the other it is interesting to note that these periods are exactly superposable and thus predictable.

6 Calendar of peak flowering

Stage 3 used for indicating spectacular or mass-flowering enables to establish a calendar with the periods of peak flowering (Appendix 6). Even if stage 3 appears only once, it has been taken into account to make this calendar. There may thus exist a discrepancy between Appendix 6 and Appendix 1 as in Appendix 1 taxa are listed only when at least five years of observations have been made. Such a calendar of peak flowering periods is useful for horticulturists, collection managers, gardeners and for the general public visiting the greenhouses.

7 Further research needed

It would be interesting to investigate the flowering and fruiting behaviour of plants grown in other botanical gardens or private collections in the world in order to evaluate their significance. What is the influence of external factors such as latitude, temperature, sunshine, photoperiodism, on the flowering / fruiting periods of the species? How do react plants grown in the northern and in the southern hemisphere? Are the duration of each stage of flowering and of fruiting identical? Is there a relation between the phenotypical behaviour in the wild and in cultivation? Certain parameters can be constant for one species and this information will enable to precise the auto-ecology of the species.

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Note added in proof

7 Further research needed: How do react plants grown in the northern and the southern hemisphere ?

A recent publication (Hallé *et al.* 2002) explains how plants originating from the southern hemisphere react when grown in the northern part of the world. Most of them do not inverse their flowering rhythm and keep their southern flowering period. Our observations confirm this. *Cunonia capensis* for example flowers during the same months in Southern Africa (spring) and in Belgium (autumn); *Haemanthus albiflos* and *Banksia integrifolia* have also this behaviour. On the other hand, some species change their flowering period and flower during springtime in their original habitat as well as in Belgium : *Crinum moorei*, *Dietes iridioides*, *Acacia dealbata* and *A. longifolia* are some examples. However no general rule can be expressed; plants belonging to the same biological form or the same taxonomical group (genus or family), or having the same geographical origin can either keep or inverse their rhythm.

Hallé F., G. Escoubeyrou, V. Mure-Bombal & B. Descoings (2002) Conservation ou abandon du rythme austral. *J. Bot. Soc. Bot. France* 18-19: 151-160.

Appendix 1.

Selection of species showing periods and types of flowering and fruiting behaviour.

–: absence of flowering or fruiting. Weeks in brackets: not frequently observed.

Families listed according to Mabberley 1997.

Species (Family)	FL weeks	FL type	FR weeks	FR type
Acmena smithii (Myrtaceae)	18-32	1-2	52-16	4-1
Aglaonema commutatum 'Tricolor' (Araceae)	24-40	2	1-52	1-2-3-4
Aglaonema nitidum (Araceae)	40-4	3-4	10-22	4-1
Alectryon subcinereus (Sapindaceae)	26-46	2-3	4-28	4-1
Ardisia compressa (Myrsinaceae)	(47)50-21	4-1	11-22	1
Asystasia vogeliana (Acanthaceae)	47-9(15)	3-4	1-15	4-1
Camellia japonica (Theaceae)	48-18	4-1	26-42	2-3
Carissa bispinosa (Apocynaceae)	2-27	4-1	1-11,29-39	5
Coffea racemosa (Rubiaceae)	9-23	1	27-31	2
Encephalartos laurentianus (Zamiaceae)	25-41	2	51-17	4-1
Eriobotrya japonica (Rosaceae)	44-5	3-4	18-34	1-2
Euclinia longiflora (Rubiaceae)	47-1	3	–	
Fuchsia procumbens (Onagraceae)	12-38	1-2	28-10	2-3-4
Haemanthus albiflos (Amaryllidaceae)	41-52(15-17)	3 (5)	7-13	4
Heliconia humilis (Heliconiaceae)	44-24	3-4-1	48-32	1-2-3-4
Laurus nobilis (Lauraceae)	1-11	4	–	
Leea coccinea (Leeaceae)	26-48	2-3	52-24	4-1
Macadamia integrifolia (Proteaceae)	44-14	3-4	1-52	1-2-3-4
Mackaya bella (Acanthaceae)	4-26	4-1	–	
Myrtus communis (Myrtaceae)	23-35	1-2	(7,8)41-49	3
Nelumbo nucifera (Nelumbonaceae)	26-39	2	34-44	2-3
Ochna atropurpurea (Ochnaceae)	5-23	4-1	23-37	1-2
Palisota bracteosa (Commelinaceae)	1-15	4	12-47	1-2-3
Pittosporum crassifolium (Pittosporaceae)	2-12	4	18	1
Pseuderanthemum longifolium (Acanthaceae)	19-38(48)	1-2	32-50(5)	2-3
Sabal minor (Arecaceae)	20-27	1	32-5	2-3-4
Triphasia trifolia (Rutaceae)	12-33(41)	1-2	1-52	1-2-3-4

Appendix 2. Types of flowering behaviour.

Observations in the greenhouses of the National Botanical Garden of Belgium.

Flowering type 1 : spring (weeks 13-25, 21 March - 20 June)

Acca sellowiana, Alocasia macrorrhizos, Brownea capitella, Caladium (Bicolor-hybrid) cultivar, *Calathea varians, Campomanesia xanthocarpa* var. *xanthocarpa, Carludovica palmata, Carmichaelia stricta, Cattleya skinneri, Cattleya trianaei* f. *concolor, Catunaregam spinosa, Cestrum foetidissimum, Chamaerops humilis* 'Tomentosa', *Chamaerops humilis, Chlorophytum stenopetalum, Chrysophyllum imperiale, Clavija longifolia, Coffea arabica, Coffea arabica* 'Polysperma', *Coffea arabica* 'San Ramon', *Coffea arabica* 'de Miberisi', *Coffea arabica* var. *abyssinica, Coffea canephora, Coffea kapakata, Coffea racemosa, Coffea x arabusta, Cordyline australis, Crinum jagus, Cyclanthus bipartitus, Dendrobium chrysotoxum, Dianella caerulea, Didymosalpinx norae, Dietes iridioides, Diospyros digyna, Dracaena fragrans, Echium hierrense, Entelea arborescens, Heliconia angusta, Lycaste candida, Magnolia liliifera* var. *liliifera, Maytenus heterophylla, Philodendron devansayeanum, Philodendron gloriosum, Philodendron pinnatifidum, Philodendron verrucosum, Philodendron wendlandii, Pittosporum ralphii, Pittosporum tenuifolium, Primula malacoides, Rauvolfia vomitoria, Rhus succedanea, Sabal minor, Schinus longifolius, Strelitzia reginae* var. *humilis, Trachycarpus fortunei*

Flowering type 2 : summer (weeks 26-38, 21 June - 20 September)

Aglaonema commutatum 'Tricolor', *Angraecum scottianum, Asparagus densiflorus* 'Sprengeri', *Asplundia* sp., *Bauhinia forficata, Billbergia pyramidalis, Cochliostema odoratissimum, Crescentia cujete, Diospyros soubreana, Encephalartos laurentianus, Erythrina crista-galli, Escallonia bifida, Francoa sonchifolia, Francoa sonchifolia* var. *ramosa, Gardenia cornuta, Haemanthus coccineus, Hedychium spicatum, Heimia myrtifolia, Houttuynia cordata, Ipomoea bonariensis, Lagerstroemia indica, Markhamia tomentosa, Maurocenia frangula, Monodora myristica, Montrichardia arborescens, Myrtus communis* var. *tarentina, Nelumbo nucifera, Phoenix roebelenii, Psychotria brasiliensis, Solanum capsicastrum*

Flowering type 3 : autumn (weeks 39-51, 21 September - 20 December)

Alpinia vittata, Angraecum eburneum subsp. *eburneum* var. *longicalcar, Angraecum leonis, Bixa orellana, Camellia japonica* subsp. *rusticana, Camellia sasanqua, Cattleya* 'Barbara', *Cattleya labiata, Chasmanthe aethiopica, Clerodendrum nutans, Dombeya tiliacea, Eriobotrya japonica* 'Don Carlos', *Euclinia longiflora, Haemanthus albiflos, Hypoestes phyllostachya, Jacobinia chrysostephana, Paphiopedilum insigne* var. *sanderae, Pilea cadierei, Plectranthus ambiguus, Ptychosperma ambiguum, Setaria palmifolia*

Flowering type 4 : winter weeks 52-12, 21 December - 20 March)

Acacia longifolia, Acacia longifolia var. *floribunda, Alocasia cucullata, Aloe arborescens* var. *ucriae, Aloe pluridens, Aloe sessiliflora, Angraecum eburneum* subsp. *eburneum* var. *longicalcar, Azara microphylla, Buxus balearica, Cattleya labiata, Crassula ovata, Cupressus lusitanica, Dracaena aubryana, Erica triflora, Eugenia uniflora, Eupatorium vernale, Heliconia bicolor, Hoffmannia ghiesbreghtii, Hoffmannia ghiesbreghtii* 'Variegata', *Laelia anceps, Laurus azorica, Laurus nobilis, Ludovia lancifolia, Mahonia napaulensis, cf. Myrciaria, Palisota bracteosa, Pandanus utilis, Paphiopedilum* 'Goultenianum' x 'Alma Gevaert', *Paphiopedilum sukhakulii, Pittosporum crassifolium, Rhamnus alaterna, Scadoxus multiflorus* subsp. *multiflorus*

Flowering type 1-2 : spring - summer (weeks 13-38)

Acmena smithii, Alpinia zerumbet, Annona cherimola, Anthurium veitchii, Antidesma bunius, Anubias heterophylla, Ardisia humilis, Asparagus falcatus, Baphia nitida, Callicarpa americana, Cattleya forbesii, Cocculus laurifolius, Coffea liberica, Cordyline rubra 'Bruantii', *Costus vargasii, Crinum moorei, Ctenanthe compressa* var. *luschnathiana, Dais cotinifolia, Echinodorus grandiflorus* subsp. *grandiflorus, Echinodorus intermedius, Echium wildpretii, Elaeodendron capense, Episcia cupreata, Escallonia x iveyi, Eupatorium sordidum, Fuchsia procumbens, Funtumia elastica, Geranium palmatum, Heteranthera reniformis, Jatropha curcas, Malpighia punicifolia, Manihot dulcis* var. *multifida, Mascarenhasia arborescens, Medinilla magnifica, Melaleuca hypericifolia, Miltoniopsis phalaenopsis, Myrtus communis, Napoleonaea vogelii, Nephthytis afzelii, Palisota albertii, Palisota alopecurus, Palisota pynaertii, Palisota pynaertii* 'Elizabethae', *Palisota schweinfurthii, Paphiopedilum parishii, Philodendron regelianum, Phoenix reclinata, Proiphys amboinensis, Pseuderanthemum longifolium, Psilanthus ebracteolatus, Psychotria peduncularis, Renanthera* *storiei, Stephanotis floribunda, Syzygium samarangense, Theobroma grandiflorum, Thunbergia erecta, Topobea parasitica, Trachelospermum jasminoides, Triphasia trifolia, Typhonodorum lindleyanum, Ziziphus spina-christi*

Flowering type 2-3 : summer - autumn (weeks 26-51)

Aeginetia indica, Aglaonema costatum 'Splendens', *Alectryon subcinereus, Alpinia purpurata, Aphelandra tetragona, Boehmeria cylindrica, Brugmansia* 'Rosa Traum', *Calathea albertii, Calathea leopardina, Callicarpa cana, Clusia ovigera, Costus malortieanus, Crinum moorei* 'Album', *Crotalaria capensis, Dichorisandra thyrsiflora, Elatostema repens* var. *repens, Etlingera elatior, Eugenia leonensis, Gloxinia perennis, Hedychium greenii, Heliconia pendula, Hevea brasiliensis, Hybanthus communis, Ipomoea mauritiana, Ixora chinensis* 'Alba', *Jacquinia aurantiaca, Leea coccinea, Leea guineensis, Leea rubra, Mimosa pudica, Myristica fragrans, Nerium oleander* 'Atropurpureum', *Ornithophora radicans, Phragmipedium besseae, Picramnia pentandra, Pilea crassifolia* 'Moon Valley', *Plumbago auriculata, Polygonum molle, Pseuderanthemum alatum, Ptychosperma schefferi, Punica granatum* 'Nana', *Quisqualis falcata* var. *mussaendiflora, Scadoxus multiflorus* subsp. *katherinae, Schinus terebinthifolius, Senna x floribunda, Tapeinocheilos ananassae*

Flowering type 3-4: autumn - winter (weeks 39-12)

Acacia dealbata, Aglaonema nitidum, Ananas bracteatus, Anthurium pedatum, Asystasia vogeliana, Camellia sinensis, Canarina canariensis, Cinchona pubescens, Debregeasia longifolia, Eriobotrya japonica, Eucharis grandiflora, Ficus lingua subsp. *lingua, Ficus religiosa, Homalomena rubescens, Jacquinia aculeata, Macadamia integrifolia, Miconia calvescens, Paphiopedilum victoria-regina, Picrasma excelsa, Prunus salasii, Ruscus aculeatus, Ruscus hypophyllum, Strophanthus preussii*

Flowering type 4-1 : winter - spring (weeks 52-25)

Acacia verticillata, Acanthus polystachyus, Aloe plicatilis, Ardisia compressa, Brownea ariza, Brownea grandiceps, Calliandra haematocephala, Callicoma serratifolia, Callistemon citrinus, Camellia japonica, Carissa bispinosa, Ceanothus thyrsiflorus 'Repens', *Cinnamomum burmanii, Clerodendrum x speciosum, Corynocarpus laevigatus, Crinum jagus, Cyperus alternifolius, Deherainia smaragdina, Dombeya wallichii, Dracaena camerooniana, Griselinia lucida, Jasminum polyanthum, Mackaya bella, Maesa argentea, Myrsine africana, Ochna atropurpurea, Osteomeles anthyllidifolia, Paphiopedilum micranthum, Persea americana, Philodendron eximium, Phoenix dactylifera, Pitcairnia corallina, Pittosporum undulatum, Prunus capolin, Reinwardtia indica, Rhaphiolepis umbellata, Rhododendron scabrum* 'Oomurasaki', *Ribes speciosum, Rosmarinus officinalis, Strelitzia reginae, Syzygium jambos, Trevesia palmata, Viburnum odoratissimum*

Flowering type 1-2-3 : spring - summer - autumn (weeks 13-51)

Acalypha sp., *Aglaia odorata, Anigozanthos flavidus, Annona montana, Aphelandra sinclairiana, Asclepias curassavica, Averrhoa carambola, Bombacopsis glabra, Brugmansia suaveolens, Callistemon citrinus, Colocasia gigantea, Crescentia cujete, Curculigo capitulata, Cyphomandra betacea, Erythrochiton brasiliensis, Eucalyptus camaldulensis, Eugenia aggregata, Fuchsia arborescens, Hemigraphis repanda, Hibbertia scandens, Hibiscus x archeri, Hoffmannia discolor, Homalocladium platycladum, Hydrocleys nymphoides, Jacobinia carnea, Jacobinia carnea* var. *velutina, Justicia spicigera, Malpighia coccigera, Medinilla myriantha, Nerium oleander* cultivar, *Nymphoides indica, Oncoba routledgei, Oncidium kramerianum, Oryza sativa* 'Bellardone', *Oxyanthus formosus, Pachira aquatica, Paphiopedilum victoria-regina, Parathesis serrulata, Paullinia pinnata, Petrea volubilis, Psidium guajava, Psidium littorale* var. *littorale, Psidium littorale* var. *longipes, Psychotria kirkii, Psychotria kirkii* var. *tarambassica, Rondeletia odorata, Ruellia blumei, Ruellia graecizans, Ruellia makoyana, Ruspolia seticalyx, Sechium edule, Solanum pseudocapsicum, Tabernaemontana coronaria* 'Flore pleno', *Tamarindus indica, Thevetia peruviana, Trichilia maynasiana* subsp. *maynasiana*

Flowering type 2-3-4 : summer - autumn - winter (weeks 26-12)

Allamanda cathartica, Alocasia x uhinkii, Banksia integrifolia, Chrysophyllum cainito, Cocos nucifera, Crinum macowanii, Cunonia capensis, Eugenia berteriana, Eulophia streptopetala, Hamelia patens, Justicia rizzinii, Ledenbergia seguierioides, Megaskepasma erythrochlamys, Osmanthus fragrans, Paphiopedilum victoria-regina, Plectranthus fruticosus, Severinia buxifolia, Tibouchina urvilleana, Viburnum tinus

Flowering type 3-4-1 : autumn - winter - spring (weeks 39-25)

Acacia farnesiana, Acokanthera oblongifolia, Callistemon salignus, Chamaedorea tepejilote, Clerodendrum splendens, Dodonaea viscosa, Echium giganteum, Elaeodendron australe, Heliconia humilis, Jasminum mesnyi, Malvaviscus arboreus, Malvaviscus arboreus var. *mexicanum, Melicope ternata, Mitriostigma axillare, Morus alba* 'Tonkin', *Palisota hirsuta, Pogostemon cablin, Sparmannia africana, Steriphoma cleomoides, Thunbergia laurifolia, Zantedeschia aethiopica* 'Devoniensis'

Flowering type 4-1-2 : winter - spring - summer (weeks 52-38)

Anthurium crassinervium, Citrus maxima, Citrus medica, Clerodendrum schweinfurthii, Clerodendrum thomsoniae, Clivia x cyrtanthiflora, Dendrobium cultivar, *Dovyalis caffra, Dypsis lutescens, Fuchsia boliviana, Hemigraphis alternata, Hoffmannia refulgens, Kigelia africana, Macleania insignis, Palisota ambigua, Paphiopedilum glaucophyllum* var. *mocquettianum, Passiflora edulis, Phyllanthus angustifolius, Psidium littorale* var. *longipes, Treculia africana, Vanilla planifolia*

Flowering type 1-2-3-4 : continuous flowering

Acacia retinodes, Acacia rubida, Anthurium cordifolium, Anthurium gracile, Anthurium lilacinum, Anthurium nymphaeifolium, Anthurium pentaphyllum var. *digitatum, Anthurium pentaphyllum* var. *pentaphyllum, Anthurium triumphans, Begonia schultzei, Begonia squamulosa, Bouvardia* 'Alba', *Brugmansia aurea, Bruguiera gymnorrhiza, Calliandra tweedii, Costus afer, Cyrtosperma johnstonii, Dactylaena micrantha, Dalechampia spathulata, Dalechampia spathulata* 'Alba', *Dischidia pectenoides, Dracaena surculosa* var. *maculata, Drimys winteri, Fuchsia microphylla, Fuchsia splendens, Garcia nutans, Glyphaea brevis, Hibiscus denisoni, Ipomoea indica, Ixora chinensis, Jasminum nitidum, Jasminum sambac, Juanulloa aurantiaca, Kopsia fruticosa, Monodora angolensis, Musa acuminata* 'Dwarf Cavendish', *Phyllanthus mimosoides, Pseuderanthemum ludovicianum, Psychotria calva, Rivina humilis, Ruellia solitaria, Salacia lehmbachii* var. *aurantiaca, Schaueria calycotricha, Scutellaria coccinea, Scutellaria costaricana, Sphyrospermum buxifolium, Syzygium paniculatum, Theobroma cacao, Thunbergia erecta* 'Alba', *Triplochlamys multiflora*

Flowering type 5 : two or more flowering periods / year

Alpinia calcarata, Anthurium colonicum, Anthurium gladiifolium, Artocarpus heterophyllus, Asplundia rigida, Brexia madagascariensis, Brosimum alicastrum, Brugmansia sanguinea, Brunfelsia americana, Cassipourea barteri, Cestrum aurantiacum, Cestrum grandiflorum, Chloranthus spicatus, Chytranthus setosus, Clausena anisata, Clinacanthus siamensis, Clivia miniata, Crinum americanum, Crinum jagus, Dracaena aubryana, Eichhornia crassipes, Ficus cf. fistulosa, Ficus natalensis subsp. *leprieurii, Ficus sur, Gossypium hirsutum, Hibiscus schizopetalus, Hymenocallis littoralis, Libertia grandiflora, Megaskepasma erythrochlamys, Monodora myristica, Monstera adansonii* var. *adansonii, Murraya paniculata, Nandina domestica, Ochna kirkii, Paphiopedilum glaucophyllum, Paphiopedilum sukhakulii, Philodendron pedatum, Phyllanthus arbuscula, Porphyrocoma longifolia, Psidium laurifolium, Ruellia devosiana, Ruellea rosea, Sanchezia nobilis, Stenospermation ulei, Terminalia catappa, Thunbergia mysorensis, Zygia latifolia* var. *latifolia*

Appendix 3. Types of fruiting behaviour.

Observations in the greenhouses of the National Botanical Garden of Belgium.

Fruiting type 1 : spring (weeks 13-25, 21 March - 20 June)

Ardisia compressa, Eugenia uniflora, Pittosporum crassifolium

Fruiting type 2 : summer (weeks 26 - 38, 21 June - 20 September)

Coffea racemosa, Francoa sonchifolia, Francoa sonchifolia var. *ramosa, Jatropha curcas, Viburnum odoratissimum*

Fruiting type 3 : autumn (week 39 - 51, 21 September - 20 December)

Hedychium spicatum, Myrtus communis, Sparmannia africana

Fruiting type 4 : winter (weeks 52 - 12, 21 December - 20 March)

Brugmansia 'Rosa Traum', *Debregeasia longifolia, Fuchsia splendens, Haemanthus albiflos, Scadoxus multiflorus* subsp. *katherinae*

Fruiting type 1-2 : spring - summer (weeks 13 - 38)

Anthurium crassinervium, Eriobotrya japonica, Eugenia uniflora, Fuchsia boliviana, Mahonia napaulensis, Ochna atropurpurea, Prunus capolin, Scadoxus multiflorus subsp. *multiflorus, Trevesia palmata*

Fruiting type 2-3 : summer - autumn (weeks 26 - 51)

Camellia japonica, Carludovica palmata, Dianella caerulea, Dischidia pectenoides, Hybanthus communis, Nelumbo nucifera, Oryza sativa 'Bellardone', *Passiflora edulis, Pseuderanthemum alatum, Pseuderanthemum longifolium, Rhaphiolepis umbellata, Ruellia blumei, Sechium edule, Syzygium samarangense, Zygia latifolia* var. *latifolia*

Fruiting type 3-4 : autumn - winter (weeks 39 - 12)

Aeginetia indica, Cestrum grandiflorum, Coffea arabica 'Polysperma', *Eugenia berteriana, Garcia nutans, Nandina domestica, Setaria palmifolia*

Fruiting type 4-1 : winter - spring (weeks 52 - 25)

Acmena smithii, Aglaonema nitidum, Alectryon subcinereus, Asystasia vogeliana, Coffea arabica, Coffea arabica 'de Miberisi', *Coffea arabica* 'San Ramon', *Encephalartos laurentianus, Leea coccinea, Prunus salasii, Ptychosperma ambiguum, Ruscus hypophyllum, Tibouchina urvilleana*

Fruiting type 1-2-3 : spring - summer - autumn (weeks 13 - 51)

Annona montana, Anthurium colonicum, Echinodorus grandiflorus subsp. *grandiflorus, Melicope ternata, Musa acuminata* 'Dwarf Cavendish', *Palisota ambigua, Palisota bracteosa, Phyllanthus arbuscula, Ruellia graecizans, Ruellia solitaria, Schaueria calycotricha, Syzygium jambos, Tamarindus indica*

Fruiting type 2-3-4 : summer - autumn - winter (weeks 26 - 12)

Asclepias curassavica, Averrhoa carambola, Clerodendrum splendens, Corynocarpus laevigatus, Erythroxylum novogranatense, Fuchsia procumbens, Jasminum polyanthum, Medinilla myriantha, Osteomeles anthyllidifolia, Pavonia spinifex, Psidium littorale var. *littorale, Ruspolia seticalyx, Sabal minor, Solanum pseudocapsicum, Syzygium paniculatum*

Fruiting type 3-4-1 : autumn - winter - spring (weeks 39 - 25)

Asparagus densiflorus 'Sprengeri', *Clausena anisata, Cyphomandra betacea, Dalechampia spathulata* 'Alba', *Heimia myrtifolia, Morus alba* 'Tonkin', *Ptychosperma schefferi, Theobroma cacao*

Fruiting type 4-1-2 : winter - spring - summer (weeks 52 - 38)

Cestrum aurantiacum, Pogostemon cablin, Prunus salasii, Vanilla planifolia

Fruiting type 1-2-3-4 : continuous fruiting

Acacia farnesiana, Aglaonema commutatum 'Tricolor', *Aglaonema costatum* 'Splendens', *Ananas bracteatus, Anthurium gracile, Anthurium lilacinum, Ardisia humilis, Bombacopsis glabra, Callistemon citrinus, Chamaedorea tepejilote, Chlorophytum stenopetalum, Citrus maxima, Citrus medica, Clerodendrum schweinfurthii, Clivia x cyrtanthiflora, Clivia miniata, Coffea liberica, Dactylaena micrantha, Dalechampia spathulata, Dodonaea viscosa, Dracaena camerooniana, Dracaena surculosa* var. *maculata, Entelea arborescens, Erythrochiton brasiliensis, Eucalyptus camaldulensis, Ficus religiosa, Gossypium hirsutum, Heliconia humilis, Macadamia integrifolia, Maesa argentea, Melaleuca hypericifolia,* cf. *Myrciaria, Nephthytis afzelii, Oncoba routledgei, Pandanus utilis, Parathesis serrulata, Phoenix dactylifera, Picramnia pentandra, Psychotria calva, Psychotria kirkii* var. *tarambassica, Ruellia rosea, Salacia lehmbachii* var. *aurantiaca, Scutellaria coccinea, Solanum capsicastrum, Sphyrospermum buxifolium, Steriphoma cleomoides, Theobroma cacao, Trichilia maynasiana* ssp. *maynasiana, Triphasia trifolia, Viburnum tinus*

Fruiting type 5 : several fruiting periods / year

Carissa bispinosa, Ficus cf. fistulosa, Ficus sur, Ochna kirkii, Porphyrocoma longifolia, Psidium guajava, Psidium littorale var. *longipes, Stenospermation ulei*

Appendix 4. Species with short flowering periods.
Observations in the greenhouses of the National Botanical Garden of Belgium.

Duration of FL	Species
1 week	*Bixa orellana*
2 weeks	*Rhus succedanea*
4 weeks	*Billbergia pyramidalis, Catunaregam spinosa, Chamaerops humilis, Chlorophytum stenopetalum, Didymosalpynx norae, Eriobotrya japonica* 'Don Carlos', *Phoenix roebelinii, Pilea cadierei*
5 weeks	*Asplundia* sp., *Campomanesia xanthocarpa, Echium hierrense, Myrtus communis* var. *tarentina*
6 weeks	*Angraecum leonis, Angraecum scottianum, Buxus balearica, Coffea arabica* (and cultivars), *Coffea x arabusta, Euclinia longiflora, Francoa sonchifolia, Pandanus utilis, Philodendron devansayanum, Philodendron gloriosum, Pittosporum tenuifolium, Proiphys amboinensis*

Appendix 5. Species with short fruiting periods.
Observations in the greenhouses of the National Botanical Garden of Belgium.

Duration of FR	Species
6 weeks	*Francoa sonchifolia, Haemanthus albiflos*
8 weeks	*Anthurium crassinervium, Brugmansia* 'Rosa Traum', *Debregeasia longifolia, Mahonia napaulensis, Myrtus communis*
9 weeks	*Viburnum odoratissimum*
10 weeks	*Fuchsia splendens, Hedychium spicatum, Nelumbo nucifera*

Appendix 6. Calendar of peak flowering.

Observations in the greenhouses of the National Botanical Garden of Belgium.

January : *Acacia dealbata, Acacia longifolia, Aloe pluridens, Ardisia compressa, Asystasia vogeliana, Camellia japonica, Clerodendrum splendens, Clinacanthus siamensis, Clivia x cyrtanthiflora, Cupressus lusitanica, Dracaena aubryana, Drimys winteri, Hoffmannia ghiesbreghtii, Justicia rizzinii, Melicope ternata, Pandanus utilis, Picrasma excelsa, Scadoxus multiflorus* subsp. *multiflorus, Trevesia palmata*

February : *Acacia longifolia, Acacia longifolia* var. *floribunda, Acacia verticillata, Acanthus polystachyus, Aloe pluridens, Camellia japonica, Citrus maxima, Clinacanthus siamensis, Clivia x cyrtanthiflora, Crinum jagus, Drimys winteri, Erica triflora, Eupatorium vernale, Hoffmannia ghiesbrechtii, Hoffmannia ghiesbreghtii* 'Variegata', *Jasminum mesnyi, Justicia rizzinii, Laurus azorica, Mahonia napaulensis, Melicope ternata, Pandanus utilis, Persea americana, Phyllanthus arbuscula, Pittosporum crassifolium, Porphyrocoma longifolia, Reinwardtia indica, Rhamnus alaterna, Rhaphiolepis umbellata, Rhododendron scabrum* 'Oomurasaki', *Ribes speciosum, Scadoxus multiflorus* subsp. *multiflorus, Scutellaria costaricana, Sparmannia africana, Steriphoma cleomoides, Syzygium jambos, Trevesia palmata*

March : *Acacia verticillata, Acokanthera oblongifolia, Alocasia macrorrhizos, Alocasia x uhinkii, Asclepias curassavica, Buxus balearica, Callicoma serratifolia, Camellia japonica, Citrus maxima, Clausena anisata, Clinacanthus siamensis, Clivia miniata, Clivia x cyrtanthiflora, Dendrobium chrysotoxum, Dombeya wallichii, Dovyalis caffra, Dracaena aubryana, Dracaena surculosa* var. *maculata, Drimys winteri, Ficus* cf. *fistulosa, Fuchsia boliviana, Fuchsia splendens, Heliconia bicolor, Hoffmannia refulgens, Hymenocallis littoralis, Jasminum polyanthum, Justicia rizzinii, Malpighia coccigera, Monodora angolensis, Persea americana, Petrea volubilis, Phyllanthus angustifolius, Pittosporum crassifolium, Pittosporum tenuifolium, Pogostemon cablin, Porphyrocoma longifolia, Primula malacoides, Prunus capolin, Reinwardtia indica, Rhododendron scabrum* 'Oomurasaki', *Salacia lehmbachii* var. *aurantiaca, Schaueria calycotricha, Scutellaria costaricana, Thunbergia affinis, Thunbergia erecta* 'Alba', *Thunbergia mysorensis, Triplochlamys multiflora, Zygia latifolia* var. *latifolia*

April : *Acca sellowiana, Acokanthera oblongifolia, Aloe plicatilis, Antidesma bunius, Brownea ariza, Callicoma serratifolia, Clavija longifolia, Clerodendrum schweinfurthii, Clerodendrum splendens, Crinum jagus, Dendrobium chrysotoxum, Dracaena aubryana, Dypsis lutescens, Echium wildpretii, Elaeodendron australe, Erythroxylum novogranatense, Eucalyptus camaldulensis, Fuchsia boliviana, Heliconia humilis, Hymenocallis littoralis, Mackaya bella, Mitriostigma axillare, Monodora angolensis, Osteomeles anthyllidifolia, Palisota pynaertii, Palisota pynaertii* 'Elizabethae', *Phyllanthus arbuscula, Pittosporum ralphii, Primula malacoides, Salacia lehmbachii* var. *aurantiaca, Schinus longifolius, Scutellaria costaricana, Thunbergia erecta* 'Alba', *Thunbergia mysorensis*

May : *Acacia retinodes, Acca sellowiana, Acokanthera oblongifolia, Asclepias curassavica, Baphia nitida, Carmichaelia stricta, Cestrum foetidissimum, Cestrum grandiflorum, Clerodendrum schweinfurthii, Clerodendrum splendens, Clivia x cyrtanthiflora, Corynocarpus laevigatus, Crinum jagus, Didymosalpinx norae, Dypsis lutescens, Echium wildpretii, Entelea arborescens, Erythroxylum novogranatense, Eucalyptus camaldulensis, Fuchsia boliviana, Geranium palmatum, Griselinia lucida, Heliconia angusta, Hemigraphis repanda, Libertia grandiflora, Mackaya bella, Macleania insignis, Mitriostigma axillare, Palisota alopecurus, Palisota pynaertii* 'Elizabethae', *Passiflora edulis, Pittosporum ralphii, Pseuderanthemum longifolium, Psidium guajava, Psychotria brasiliensis, Salacia lehmbachii* var. *aurantiaca, Strelitzia reginae, Theobroma cacao, Theobroma grandiflorum, Thunbergia affinis, Trachelospermum jasminoides, Trachycarpus fortunei, Zantedeschia aethiopica* 'Devoniensis'

June : *Acacia retinodes, Acacia rubida, Acmena smithii, Acokanthera oblongifolia, Asclepias curassavica, Asparagus falcatus, Baphia nitida, Brugmansia* 'Rosa Traum', *Brugmansia suaveolens, Callistemon citrinus, Cestrum grandiflorum, Cinnamomum burmanii, Clerodendrum thomsoniae, Clivia x cyrtanthiflora, Cocculus laurifolius, Crinum moorei* 'Album', *Dais cotinifolia, Eichhornia crassipes, Ficus* cf. *fistulosa, Geranium palmatum, Heliconia angusta, Ixora chinensis, Jacobinia carnea* var. *velutina, Justicia spicigera, Medinilla magnifica, Megaskepasma erythrochlamys, Melaleuca hypericifolia, Nandina domestica, Pachira aquatica, Palisota alopecurus, Palisota pynaertii* 'Elizabethae', *Parathesis serrulata, Petrea volubilis, Philodendron* *regelianum, Phoenix reclinata, Phyllanthus arbuscula, Polygonum molle, Pseuderanthemum longifolium, Renanthera storiei, Saxifraga stolonifera, Senna x floribunda, Theobroma cacao, Trachelospermum jasminoides, Vanilla planifolia*

July : *Acacia retinodes, Agapanthus campanulatus, Asparagus falcatus, Baphia nitida, Bauhinia forficata, Bouvardia* 'Alba', *Brugmansia* 'Rosa Traum', *Brunfelsia americana, Cassipourea barteri, Cestrum aurantiacum, Citrus medica, Clerodendrum speciosissimum, Crescentia cujete, Crinum moorei, Erythrina crista-galli, Erythroxylum novogranatense, Etlingera elatior, Eupatorium sordidum, Francoa sonchifolia, Heterocentron elegans, Ixora chinensis, Jacobinia carnea, Jacobinia carnea* var. *velutina, Markhamia tomentosa, Medinilla myriantha, Medinilla sedifolia, Megaskepasma erythrochlamys, Parathesis serrulata, Phoenix roebelinii, Polygonum molle, Pseuderanthemum longifolium, Punica granatum* 'Nana', *Renanthera storiei, Rondeletia odorata, Ruspolia seticalyx, Senna x floribunda, Tabernaemontana coronaria* 'Flore pleno', *Tamarindus indica, Theobroma cacao, Thunbergia affinis, Thunbergia erecta* 'Alba'

August : *Aeginetia indica, Aglaia odorata, Asclepias curassavica, Averrhoa carambola, Bauhinia forficata, Boehmeria cylindrica, Brugmansia* 'Rosa Traum', *Cestrum aurantiacum, Clausena anisata, Coffea liberica, Crinum moorei, Cunonia capensis, Diospyros soubreana, Encephalartos laurentianus, Erythrochiton brasiliensis, Erythroxylum novogranatense, Etlingera elatior, Ficus natalensis* subsp. *leprieurii, Fuchsia arborescens, Fuchsia splendens, Hevea brasiliensis, Homalocladium platycladum, Ixora chinensis* 'Alba', *Jacobinia carnea, Malpighia coccigera, Markhamia tomentosa, Medinilla myriantha, Philodendron pedatum, Plumbago auriculata, Polygonum molle, Psilanthus ebracteolatus, Punica granatum* 'Nana', *Ruellia graecizans, Ruspolia seticalyx, Scadoxus multiflorus* subsp. *katherinae, Senna x floribunda, Solanum capsicastrum, Tamarindus indica, Theobroma cacao*

September : *Aglaonema costatum* 'Splendens', *Alectryon subcinereus, Aphelandra tetragona, Asclepias curassavica, Averrhoa carambola, Billbergia pyramidalis, Boehmeria cylindrica, Brunfelsia americana, Calliandra tweedii, Cassipourea barteri, Crinum macowanii, Crotalaria capensis, Dalechampia spathulata, Dalechampia spathulata* 'Alba', *Dichorisandra thyrsiflora, Diospyros soubreana, Dracaena aubryana, Drimys winteri, Encephalartos laurentianus, Eucharis grandiflora, Eugenia berteriana, Fuchsia splendens, Gloxinia perennis, Hevea brasiliensis, Hymenocallis littoralis, Ipomoea bonariensis, Jacquinia aurantiaca, Maurocenia frangula, Medinilla myriantha, Mimosa pudica, Murraya paniculata, Osmanthus fragrans, Plectranthus ambiguus, Plectranthus fruticosus, Plumbago auriculata, Ruellia graecizans, Scadoxus multiflorus* subsp. *katherinae, Schinus terebinthifolius, Scutellaria costaricana, Senna x floribunda, Tabernaemontana coronaria* 'Flore pleno', *Theobroma cacao*

October : *Brugmansia* 'Rosa Traum', *Brugmansia aurea, Brugmansia suaveolens, Calliandra tweedii, Crotalaria capensis, Cyrtosperma johnstonii, Dichorisandra thyrsiflora, Dombeya tiliacea, Drimys winteri, Fuchsia splendens, Ixora chinensis* 'Alba', *Jasminum nitidum, Miconia calvescens, Mimosa pudica, Phyllanthus arbuscula, Pseuderanthemum ludovicianum, Quisqualis falcata* var. *mussaendiflora, Ruellia devosiana, Ruellia makoyana, Ruspolia seticalyx, Schinus terebinthifolius, Scutellaria costaricana, Syzygium jambos, Tibouchina urvilleana*

November : *Alocasia x uhinkii, Ananas bracteatus, Anthurium pedatum, Camellia japonica* subsp. *rusticana, Camellia sasanqua, Camellia sinensis, Cestrum aurantiacum, Clerodendrum nutans, Dalechampia spathulata, Dalechampia spathulata 'Alba', Dichorisandra thyrsiflora, Drimys winteri, Echium giganteum, Fuchsia boliviana, Fuchsia splendens, Haemanthus albiflos, Jacobinia carnea* var. *velutina, Malvaviscus arboreus* var. *mexicanum, Phyllanthus mimosoides, Plectranthus ambiguus, Renanthera storiei, Ruellia devosiana, Ruellia makoyana, Scutellaria costaricana, Sparmannia africana, Tibouchina urvilleana*

December : *Artocarpus heterophyllus, Asystasia vogeliana, Cestrum grandiflorum, Clerodendrum splendens, Cyperus alternifolius, Dracaena aubryana, Dracaena surculosa* var. *maculata, Eucharis grandiflora, Euclinia longiflora, Ficus* cf. *fistulosa, Fuchsia boliviana, Hoffmannia ghiesbreghtii* 'Variegata', *Justicia rizzinii, Malvaviscus arboreus* var. *mexicanum, Miconia calvescens, Plectranthus ambiguus, Prunus salasii, Pseuderanthemum ludovicianum, Strophanthus preussii, Syzygium jambos*

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Biodiversity and ecophysiology of Moroccan urban woody species. A case study: the trees of Fez

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Abstract. *– In the context of ongoing international scientific interest in urban environment, this work presents the main botanical and ecophysiological traits of the trees recorded in Fez, a millennial inland city of Morocco. A total of 35 genera were identified with a high specific diversity. The species listed are mostly exotic angiosperms, belonging to 40 families, dominated by Leguminosæ, Rosaceæ, Moraceæ, Palmæ, and Rutaceæ; the remaining families being represented by less than four species, each one. Among the trees occurring in Fez, evergreen and deciduous species are equally represented. A large diversity of leaf shape was observed, but simple leaves with large blades and entire margins were predominant. The presence of shade and sun leaves was observed in more than half of 48 species examined. In shade leaves, values of leaf area, relative water content, and total chlorophyll content were significantly higher than in sun leaves; whereas chlorophyll a:b ratio was lower than in sun leaves. In the same group of species, the SLA (specific leaf area) was greatly variable, but the mean value in deciduous species was twofold compared to evergreen ones. Concerning the spatial arrangement of xylem and phloem within leaf veins, the two main patterns observed are collateral (64% of species number) and amphicribral (32%). The localisation of stomata in both adaxial and abaxial epidermis occurred in more species than the sole abaxial localisation. These results are discussed with special emphasis on the main leaf traits reflecting the acclimation strategies of trees to different environmental constraints. On the whole, the study is presented as a prelude to the research activities that may both benefit from and be beneficial to the future Fez botanic garden, which is in the process of being established and which requires scientific and financial international collaboration.*

Key words*: urban trees, Fez, Morocco, evergreen, deciduous, angiosperms, leaves form and anatomy, specific leaf area, stomatal distribution, vascular patterning, relative water content, chlorophyll content, chlorophyll a:b ratio, Fez botanic garden.*

Abbreviations*: DW, dry weight; LA, leaf area; SLA, specific leaf area; RWC, relative water content; SEM, standard error of the mean.*

Introduction

Botanical, ecological and physiological studies are being increasingly applied to urban environment as fundamental tools for landscaping, urban planning and plant heritage conservation

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(Berrang & al. 1985; Kjelgren & al. 1992; Welsh 1994; Close & al. 1996; Schwets & Brown 2000).

In this context, a good deal of the work in our lab has been devoted to the description of urban vegetation in Morocco, using a combination of the above mentioned approaches. This communication summarizes the results obtained particularly on the trees of Fez City. The tree survey covered all the woody planted area of both the ancient city, the Madina, founded around the year 800 AD and registered as a universal heritage by the UNESCO (1976), and the new city in continuous development since the beginning of the last century.

Historically, before the publication of the preliminary results of this study (Benamar & al. 1996), the only available botanical report on Fez and its region, to our knowledge, goes back to 1585; it was written by a scientist from Fez, Al-Wazir Al-Ghassani, renowned botanist, doctor and minister of that time. The title of the Arabic manuscript could be translated as: *garden of flowers for the description of simples and drugs* (Al-Wazir Al-Ghassani 1585). The manuscript, still well preserved at the library of the millennial Al-Quaraouiyine University of Fez, described 380 medicinal plants in current usage in Fez, giving their origins, and explaining their appropriate method of use. The great lack of exhaustive botanical information on Fez since several centuries has been one of the main motivations of the present work. The study consisted not only in the exhaustive inventory of the local tree species, but also in the investigation of some of their specific ecological features through the characterisation of their main photosynthetic organs i.e. the leaves, by morphological, anatomical and biochemical approaches. Also, the specific traits of sun and shade leaves were compared in the tree species bearing both types of leaves in more or less sun-exposed parts of the canopy. For this purpose, leaf size, specific leaf area (SLA), relative water content, total chlorophyll concentration and chlorophyll *a*:*b* ratio were determined. For anatomical characterisation of the leaves, the dorsiventral stomatal distribution and the transectional vascular system pattern (spatial arrangement of xylem and phloem within each leaf vein) were examined. On the whole, these investigations wanted to contribute to our knowledge of the taxonomic, morphological and ecophysiological diversity of the tree species occurring in Fez. Furthermore, our interest in presenting this paper at BEDUCO II and EuroGard III Congresses has been stimulated also by recent progress in the achievement of the ambitious project of the long-awaited Botanical garden of Fez (Benamar & al. 1996). The description of the situation of woody vegetation in Fez, by various multidisciplinary indicators, is expected to be useful before the introduction of new Moroccan native and exotic tree species for *ex situ* conservation and/or education in the garden. The informal presentation and discussion of the Fez Botanic Garden project with the participants to these two Congresses was considered helpful in creating an international collaboration around the project. The desirable partnerships would be with both individual botanic gardens from Europe and international botanic gardens networks such as BGCI (Botanic Gardens Conservation International), ABGN (African Botanic Gardens Network) and JBF (French-speaking Botanic Gardens Network).

Material and methods

Geography. Fez is an inland city of the north of Morocco, characterised by: Latitude: 34° 02' N, Longitude: 5° W, Altitude: 378 to 262 m above the sea, unimodal annual rainfall: 500 mm, average winter temperature: 11°C / 13°C (minimum: 0°C), average summer temperature: 21°C / 27°C (maximum: 44°C).

Study sites. The surveys have been carried out in both ancient and new parts of the city focusing particularly on woody parks, public gardens, private gardens and patios courtyards, and along roads, avenues and streets.

Species identification. The binomial botanical name of the recorded tree species and their respective families were determined. Nomenclature follows dendrological work of Bossard & Cuisance (1984). In addition, a local field guide illustrated by colour photographs and containing clear and detailed descriptions, was set up (data not shown).

Species list. A complete list of urban tree species of Fez and corresponding families recorded in this work can be provided upon request to the corresponding author.

Sexual reproduction potential. During the last decade, numerous trees for each of all the species occurring in Fez were regularly checked for their sexual reproduction potential. Visual estimation of the frequency and abundance of flower, fruit and seed production by the tree approximately assessed this potential.

Selected species for leaf traits determination. The determination of the basic morphological, anatomical and ecophysiological leaf traits was conducted on a group of 48 species of mainly broad-leaved trees. Species with palmate and needle-shaped leaves were not examined because of the difficulty to determine some of their leaf traits such as leaf area for instance. Nevertheless, the species investigated were belonging to 21 of the 40 families recorded in Fez, including all the most represented families in the city, with the exception of the family Palmæ. The studied leaf features included characteristic leaf shape, leaf bicoloration, lamina area, specific leaf area (SLA), relative water content, mass- or leaf area-based total Chlorophyll concentration, chlorophyll *a:b* ratio, transectional vascular pattern and stomatal distribution.

Leaf sampling. For leaf traits determination, several healthy leaves, selected as representative for each studied species, were collected from the urban trees of different parts of the city from March to May 2001. For the species presenting apparent differences between the less sun-exposed leaves of the tree (shade leaves) and the most sun-exposed ones (sun leaves), both leaves types were collected from their specific location in the canopy. The leaves sampled were immediately placed in plastic hermetic box to avoid dehydration and rapidly transferred to the laboratory for the determination of their features.

Leaf shape and bicoloration. Leaf shape (entire or lobed (simple), compound, needled or palmate), leaf inclination and the occurrence of leaf bicoloration (abaxial (lower) side being more light coloured than adaxial (upper) side) were assessed by visual inspection of several ($N = 20$) leaves of four individual plants for all species recorded.

Relative water content. The leaf dry matter was determined after drying for 72 h at 80°C. Leaf relative water content was calculated as the percentage of the difference between fresh and dry weight (DW) to the fresh weight.

Leaf size and SLA. For the leaf area determination, paper photocopies of the blade of numerous (N = 10-15) healthy and fully expanded leaves of each tree species were weighted and the surface was calculated on the basis of the specific weight ($g.cm^{-2}$) of the used paper. Characteristic leaf area (cm^2) of each species was determined as a mean of the area of the N sampled leaves. Specific leaf area ($cm^2.g^{-1}$) was calculated as a ratio of the blade surface of the N sampled leaves to their total dry weight (DW).

Vascular, stomatal and mesophyll pattern. For the anatomical examination of the leaf vascular tissues and stomata, and of the presence of palisade cell layers (adaxial and/or abaxial), semithin transverse sections of the leaves were made, and then prepared by standard procedures for light microscopic observation. After the identification of the different tissues and structural elements, diagrammatic drawings were realised for the observed section, using standard symbols referring to the different anatomical elements (data not shown). A particular attention was paid to the dorsiventral distribution of the stomata, in order to identify amphistomatous (with abaxial and adaxial stomata localisation) and hypostomatous (with only abaxial stomata localisation) species. On the other hand, the relative arrangement of leaf xylem and phloem was used as a criterion for the characterisation of the tree species according to their transectional vascular pattern. Thus, four patterns were observed, i.e. collateral (xylem and phloem toward adaxial and abaxial leaf sides, respectively), bicollateral (a xylem band sandwiched between two phloem half-disks directed toward both leaf sides), amphicribral (a phloem ring surrounding the central xylem disk), and amphivasal (a xylem ring surrounding the phloem central disk).

Chlorophyll concentration. The chlorophyll concentration was determined as a mean of four measurements carried out on healthy, mature leaves of each tree species. Thus, for deciduous and evergreen species, only non-senescent leaves and current-year leaves were sampled, respectively. Six circular disks, each 6 mm in diameter, were punched from the leaf and placed immediately into 3 ml of 80 % acetone for pigment extraction. Absorbance of the clear filtered extract were recorded at 645, 663 and 652 nm, allowing the calculation of the concentrations of chlorophylls a, b and $a + b$, taking in account the total leaf disks area and DW as respective basic units. Also, the ratio of chlorophyll a to chlorophyll b (chlorophyll *a:b* ratio) was calculated.

Results and discussion

Floristic data. The surveys carried out during the last decade showed a substantial specific diversity among the trees of Fez (Benamar & al. 1996). The species listed are mainly angiosperms (fig. 1A), belonging to 40 families (fig. 2) dominated by Leguminosæ (Papilionoideæ, Mimosoideæ and Caesalpinioideæ; 13 % of the species recorded), Rosaceæ (10 %), Moraceæ (9 %), Arecaceæ (Palmæ; 7 %), and Rutaceæ (5 %); the remaining families being represented by less than three species (≤ 4 %), each one.

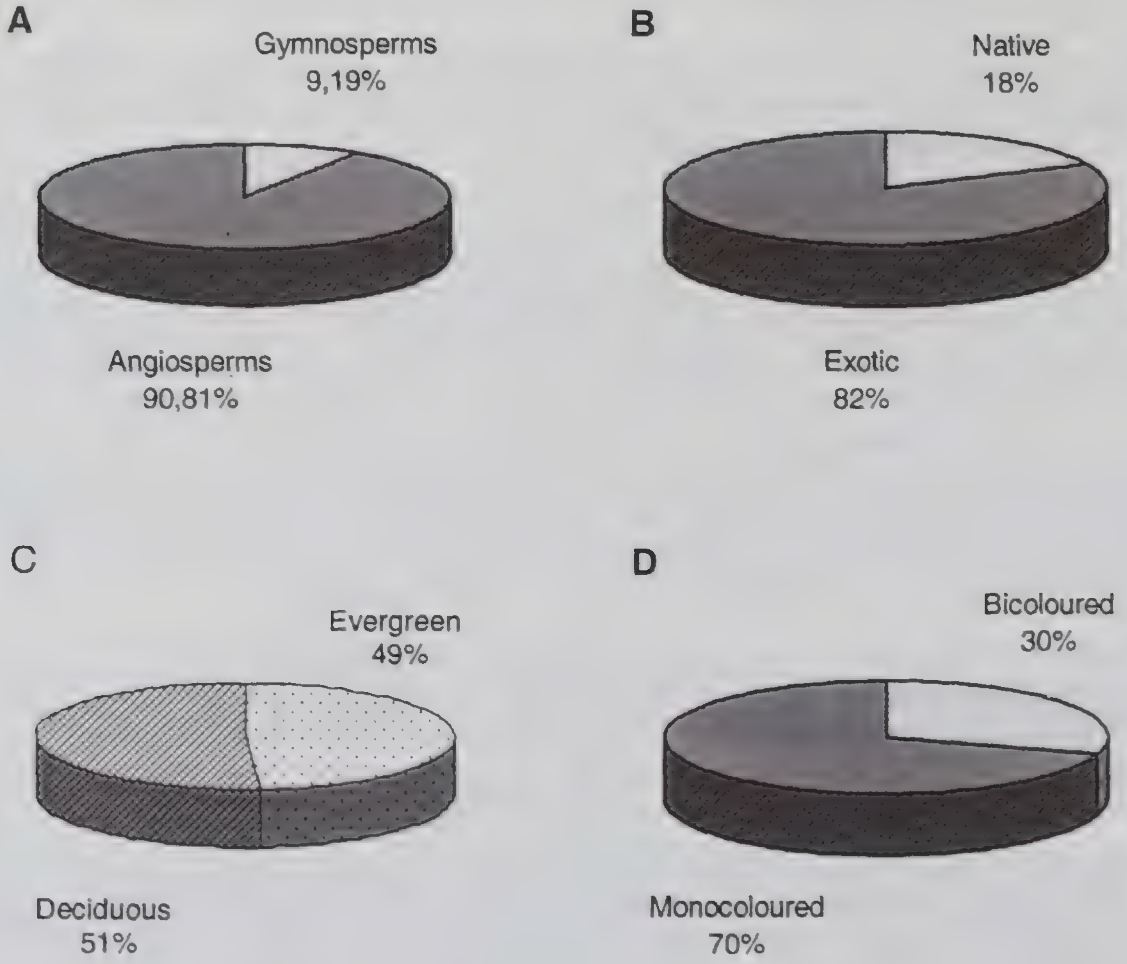


Figure 1. Distribution of the tree species recorded at Fez city in surveys carried out annually between 1994 and 2003 among: A, angiosperms and gymnosperms; B, native and exotic; C, deciduous and evergreen; D, species with bicoloured and monocoloured leaves.

Among a total of 73 genera recorded in Fez, more than half (58 %) were represented by only one individual species. The highest species richness occurred in the genera *Ficus* (5 species: *carica*; *elastica*; *benjamina*; *rubiginosa* and *retusa*) and *Citrus* (5 species: *aurantium*; *sinensis*; *nobilis*; *limonum* and *paradisii*) followed by the genera *Acacia* (4 species: *cyanophylla*; *horrida*; *cyclops* and *mollisima*) and *Prunus* (4 species: *armeniaca*; *amygdalus*; *cerasifera*; *insititia*). The majority (82 %) of the trees found in Fez are exotic species (fig. 1B), originating from the five continents. Thus, only four species were native among the four most species rich families (i.e. Leguminosæ (0 species), Rosaceæ (2), Moraceæ (1) and Arecaceæ (1)). Some of the exotic species have been introduced in Morocco many centuries ago and became spontaneous, e.g. *Morus alba*, *M. nigra*, *Zizyphus jujuba* and *Phoenix dactylifera*. Other species were brought from Australia in the beginning of the last century and have been widely planted in Morocco, such as species of the genera *Eucalyptus*, *Casuarina* and *Acacia*. A third category contains several ornamental trees, which were introduced in Fez nearly 25 years ago, e.g. *Erythrina speciosa*, *Brachychiton populneus* and *B. acerifolium*. Although a large percentage of the tree species recorded in Fez are exotic, they were in families that are mostly (65 %) part of the 131 families known for presenting one or more native plant species in Morocco (Fennane & al. 1987). Besides, more than half (55.5 %) of the total number of gymnosperms recorded in Fez were Moroccan native tree species. The number of specimens per tree species occurring in Fez is greatly variable independently of the native or exotic origin of the species.

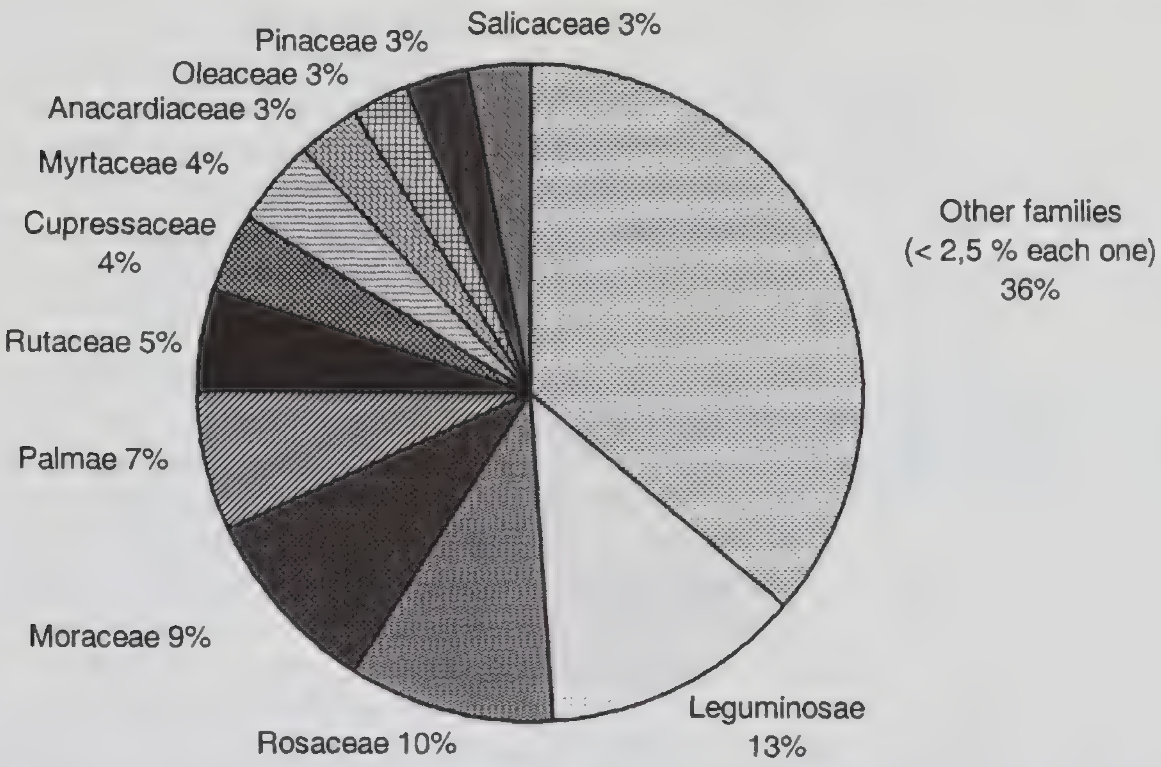


Figure 2. Distribution of the tree species recorded at Fez city in surveys carried out annually between 1994 and 2003 among different botanical families. Papilionoideæ, Mimosoideæ and Caesalpinioideæ were included in Leguminosæ. The percentages represent the relative species-richness of each family compared to the total number of species recorded.

The numerically most represented species are used either as an avenue/street tree (e.g. *Platanus acerifolia, Jacaranda mimosifolia, Phoenix canariensis, Ficus rubiginosa, Fraxinus excelsior, Sophora japonica, Morus nigra, Citrus aurantium*) or as dominant species in forest parks (e.g. *Pinus halepensis, Casuarina cuninghamiana, Eucalyptus gomphocephala, E. citriodora*) or in urban peripheral agro-forestry sites (e.g. *Olea europea, Eriobotrya japonica*). In this group there is only one native species i.e. *Olea europea* which is furthermore represented by an attractive, several hundred years old specimen situated near Bab Boujloud, one of the main gates of the historic walls of the ancient Madina of Fez. This group includes also one subspontaneous species i.e. *Morus nigra.* The second group includes less frequent species which are all exotic used as a street tree (e.g. *Lagerstroemia indica, Schinus molle*), windbreaks (e.g. *Cupressus arizonica*), a garden tree (e.g. *Citrus limonum, C. sinensis, Cydonia vulgaris*) or a specimen tree (e.g. *Cocos romanzoffiana, Salix babylonica*). The last group, including species represented by rare (one to ten) individuals, is composed by both native (e.g. *Argania spinosa, Cedrus atlantica, Tamarix aphylla, Chamerops humilis*) and exotic (e.g. *Cycas revoluta, Erythrina speciosa, Persea gratissima, Grevillea robusta, Tilia tomentosa, Koelreuteria paniculata, Ficus elastica, F. benjamina, Aesculus hippocastanum*) species. From the above-mentioned examples of species recorded in Fez, it appears that both native and exotic species are originating from a wide range of natural habitats and climates.

But, it is clear that the trees have found in Fez ecological conditions favourable to their growth and development because they appeared healthy and showed normal growth. Moreover, the mature trees of all species blossom abundantly each year, their flowering times being well displayed over the four seasons. Also, the trees produce a large quantity of fruits and seeds. The only one exception was observed in the case of mature trees of the exotic species *Araucaria excelsa,* which rarely produced cones although their growth was fast and their height was relatively high (15-20 m). On the other hand, valuable information concerning the historic changes of the tree's specific diversity in Fez could be deduced from the comparison between the results of our survey and the tree species listed in the above-mentioned work of Al-Wazir Al-Ghassani

(1585). Among a total of 67 tree species described in this manuscript because Fez inhabitants used them for medicinal purposes, 33 have been seen by the author at different precise locations in Fez. But, in this work, no data concerning the other tree species present at Fez, but without local medicinal use at that time, were mentioned. Thus, the determination of an eventual tree species diversity enrichment in Fez since then was not possible. Conversely, compared to our tree survey, the species list of Al-Wazir Al-Ghassani (1585) showed that at least five tree species disappeared from Fez since the 16th century. The species in question were both native (*Arbutus unedo, Prunus avium*) and exotic (*Citrus medica, Lycium chinense* and *Corylus avellana*) in Morocco.

Phenology. The tree species occurring in Fez were equally distributed between evergreen and winter (low temperature and high humidity) deciduous guilds (fig. 1C). Thus, none of the recorded species was drought-deciduous. The majority of the species remaining in leaf for the whole year belonged to angiosperms (40.8 % of the total species recorded) and not to gymnosperms (9.18 %). In these evergreen species, the long-lived leaves develop and expand during the warm spring and summer months and are subsequently retained and maintained during the winter months and the following growth season. In contrast to this, all the short-lived leaves in deciduous species are loosed for at least one but usually two to four months of each year. In these species, the leaf flushing (i.e. the emergence and subsequent expansion of leaves from leaf buds) occurred mostly during the spring season, leaf growth ceasing late in the dry season. Within this category, no gymnosperm tree species was recorded in Fez. On the other hand, comparing separately exotic and native species, the percentage of evergreen species appeared higher among native species (67 %) than among exotic ones (44 %). This difference might be related to the adaptation of native species to the local climate characterised by marked rainfall seasonality that causes a great variation in soil moisture content, with the peak in the wet season and the lowest level by the end of the dry season. In fact, the tendency of evergreen species to maintain a more positive turgor potential at lower values of leaf water potential (Ψ_w) compared with deciduous species, has been well documented (Sobrado 1986; Medina & Francisco 1994; Prior & al. 1997). This functional feature allows evergreen species to maintain greater cellular function at lower soil water potentials, and hence to adapt to drought conditions, more efficiently than deciduous species.

Leaf shape, bicoloration and mesophyll pattern. The leaves of all the tree species recorded in Fez were sampled for the determination of the leaf morphological features. The study showed a large diversity of leaf shape (fig. 3), with a domination of simple leaves with large blades (61 % of the total species) either lobed (11 %) or with entire margins (89 %). The remaining species possessed either compound (20 % of the total species), needled (11 %) or palmate (8 %) leaves. The phyllodes (linear to lanceolate leaf equivalent photosynthetic structure) occurring in few species such as *Acacia cyanophylla* were not counted separately but were considered as simple leaves. Also, leaves with particularly complex forms, which have been encountered in species such as *Tetraclinix articulata* and *Casuarina cuninghamiana,* were considered as needled leaves.

The trees were also surveyed for difference in another leaf feature, i.e. the presence of leaf bicoloration (adaxial side being darker than abaxial side). In this type of leaves, the lighter abaxial surface may reflect light from the spongy mesophyll side resulting in highest light retention inside the leaf (Smith 1981; Lin & Ehleringer 1983). Besides, it is considered that leaf bicoloration is associated with a more homogeneous mesophyll anatomy (no or few adaxial palisade layers), leading to a maximum light capture because the internal light is scattered by the spongy mesophyll (Knapp & al. 1988). These leaf structural properties may have an important impact on functional adaptation of species naturally occupying low light habitats, such as an understory, trough a maximisation of photosynthesis per unit leaf biomass (Smith & al. 1977). In our survey of all the tree species recorded, 30 % possessed bicoloured leaves (fig. 1D). The bicolouration occurred mainly in simple-leaved species (86.7 % of the total bicoloured leaves) and was the most marked (greatest colour difference between abaxial and adaxial leaf sides) in some of these species.

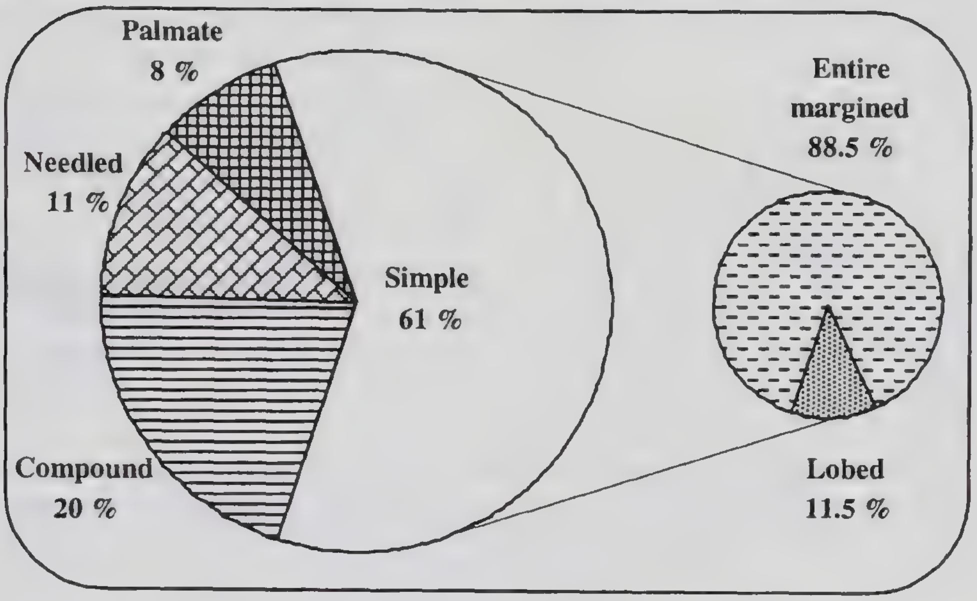


Figure 3. Relative occurrence of the diverse leaf shapes observed in the tree species recorded at Fez city in surveys carried out between 1994 and 2003. Values indicate the percentages of species sharing a given leaf shape.

In accordance with the above-mentioned general structural features associated with leaf bicoloration, our anatomical examinations showed that most of the bicoloured leaves (84 %) had a homogenous mesophyll, corresponding to dorsiventral palisade distribution. The remaining species (16 %) were isobilateral, with a well-developed palisade tissue (numerous palisade cell layers beneath both leaf surfaces). Isobilaterality and bicolouration being considered as typical of plants originating from high lighted and shaded habitats, respectively (Pyykkö 1966; Burrows 2001), a question arose relative to the signification of the unusual association of both leaf features in these few species. Nevertheless, it is noteworthy that these species (e.g. *Schinus molle*, *Ficus carica* and *Morus nigra*) were weakly bicolour (the difference between both leaf sides colours being slight). Otherwise, species with bicoloured leaves were more exotic (76 %) than native (24 %); but leaf bicolouration appeared more frequent in Moroccan native species (39 % of the total number of recorded native species) than in exotic (28 % of the total number of the exotic species recorded). On the assumption that leaf bicolouration is typically characteristic of species from shaded natural habitats, the above mentioned percentages might reflect the high diversity of Moroccan native or exotic communities from which the tree species occurring in Fez were introduced. In addition, the dominant occurrence of non-bicoloured leaves in both native (61 %) and exotic (72 %) recorded species, probably implies that the historic and recent successive introductions of species in Fez were mostly from sunny and possibly dry habitats. This is confirmed at least for relatively recent introductions, carried out since the middle of the last century, which concerned mostly high-sun tree species from Australia (Benabid 1987).

Vascular and stomatal patterning. The spatial arrangement of the two vascular tissues, xylem and phloem, within major leaf veins reflect the degree of dorsiventrality expressed by the leaf (Dengler & Kang 2001). Vascular pattern has been tightly linked to the leaf shape throughout the evolutionary history of land plants. Furthermore, leaf primary morphogenesis coincides temporally with the appearance of major veins (Nelson & Dengler 1997; Sieburth 1999) and both aspects of leaf development appeared mainly regulated by common mechanisms such as auxin (Mattson & al. 1999). Thus, the leaf vascular tissues arrangement was chosen to complete the characterisation of leaf morphology and anatomy in the Fez tree species sampled. In a group of 48 species examined, the light microscopic anatomical examination of semithin transverse sections of the leaves, showed three transectional patterns (fig. 4A); among them (fig. 4B), collateral (generally considered as typical pattern) occurred more frequently (63.8 % of the 48 species studied) than amphicribral (31.9 %) and bicollateral (4.3 %).

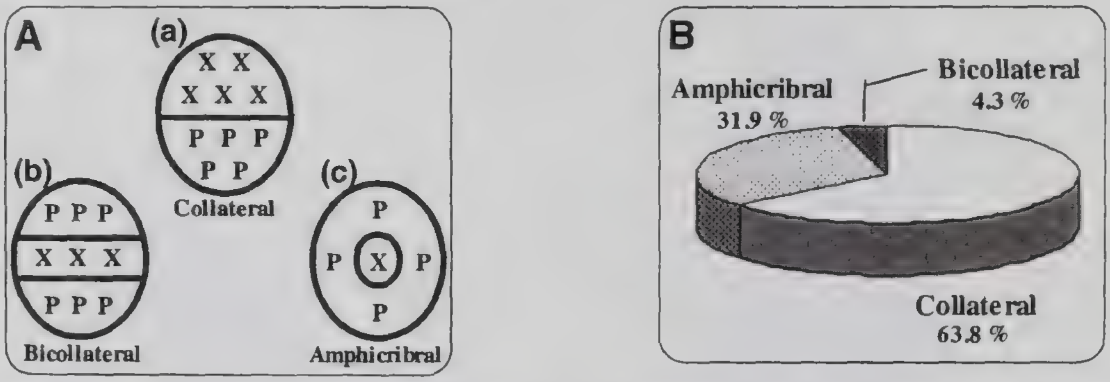


Figure 4. Vascular patterning in 48 broad-lamina leaf tree species sampled among the trees of Fez city. A, illustration of the transectional vascular patterns related to the arrangement of xylem (X) and phloem (P) tissues within a vein: (a) collateral pattern, (b) bicollateral pattern, (c) amphicribral pattern; B, percentages of the species having a given transectional vascular pattern.

None of the species examined was presenting the amphivasal pattern, in which the xylem is entirely surrounding the phloem. Thus, a high proportion of the tree species recorded possessed leaves with the typical dorsiventral symmetry, xylem and phloem being toward adaxial and abaxial leaf surfaces, respectively. Concerning the stomatal distribution between the opposite leaf sides, the presence of stomata was observed in either both adaxial and abaxial epidermis (amphistomatous species) or in only the abaxial epidermis (hypostomatous species); but, as shown in fig. 5, amphistomatous species were more frequent (61.7 %) than hypostomatous (38.3 %). The amphistomatous condition was shared by both monocotyledonous (e.g. members of the Arecaceæ family) and dicotyledonous species (belonging to numerous families). This type of stomatal distribution was generally associated with mesophyll isobilaterality and great sunlight exposure (Smith & al. 1998; Burrows 2001). The distribution of stomata on both leaf sides can result in a substantial increase of the availability of CO_2 for the well sun-exposed and developed isobilateral mesophyll tissue (Parkhurst 1994). The structural association between isobilaterality and amphistomy was confirmed in 64.3 % of the isobilateral species occurring in the group of 48 species examined. In contrast, the remaining isobilateral species (i.e. *Ficus carica*, *Morus alba*, *Morus nigra*, *Persica vulgaris* and *Schinus terbenthifolius*) had stomata only on the abaxial leaf surface. On the other hand, in the group of leaf bicoloured species, nearly 60 % were hypostomatous, confirming the admitted association between leaf bicoloration, typical of naturally light-limited species, and a hypostomatous condition.

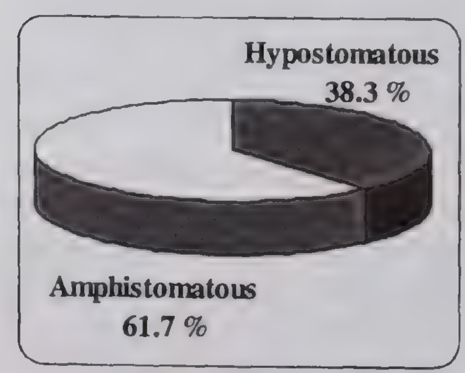
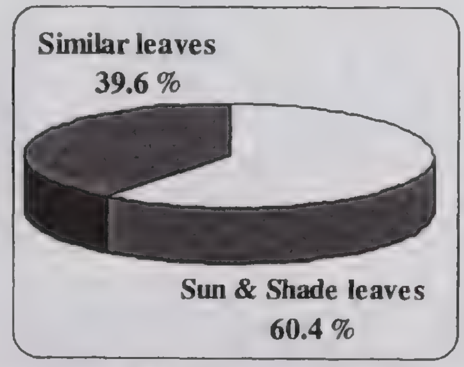


Figure 5. Stomatal patterning in 48 broad-lamina leaf tree species sampled among the trees of Fez City. Values indicate the percentages of amphistomatous species (stomata localised beneath both adaxial and abaxial leaf surfaces) and hypostomatous species (stomata localised beneath the abaxial leaf surface).

This association has been explained by the fact that in the bicoloured leaves, with the supposed light-limited photosynthetic rates, the total amount of CO_2 required can probably be supplied by stomata only on the abaxial surface (Smith & al. 1997). Stomatal distribution has been also associated with another important leaf feature i.e. leaf inclination, which is related to the level of sun-exposure and in return has a strong influence on the level of incident light (Terashima & Hikosaka 1995; Vogelmann & al. 1996). Inclined leaf form may be particularly adaptive to sun-exposed habitats. The change in leaf orientation in response to greater sun exposure enables greater photosynthesis per unit leaf biomass via the development of both a greater mesophyll cell surface area (isobilaterality) and a greater number of stomata, distributed on both leaf surfaces (amphistomy), for more efficient CO_2 absorption (Smith & al. 1998). As leaf orientation becomes more inclined with greater sun-exposure, more equal amounts of sunlight strike both leaf surfaces but adaxial and abaxial incident sunlight is substantially reduced, avoiding the photoinhibitory effect of excessive sunlight on photosynthesis (Smith & al. 1998; Baker & Bowyer 1994). The alteration of incident light, by the change of the leaves orientation represents a plant response to high light exposure, considered as sun-avoidance adaptation (Gamon & Pearcy 1989) in opposition to shade avoidance response (Smith 1982). In the survey carried out at Fez, a broad range of leaf orientation properties (from nearly horizontal to vertical) was observed. Vertical leaf orientation occurred in several species (i.e. *Eucalyptus citriodora*, *Acacia cyanophylla*, *Salix babylonica*, *Schinus molle*, *Celtis australis* and *Brachychiton populneus*) from diverse families. The structural properties relative to isobilaterality and amphistomy were shared between all these species with the exception of *Celtis australis* and *Brachychiton populneus*, in which amphistomy was associated with dorsiventrality. Such unusual mesophyll dorsiventral pattern in an overstory species has been already reported in the case of *Brachychiton populneus* (Burrows 2001). Otherwise, the association between dorsiventrality and amphistomy was observed in several species with non-vertical but strong inclined leaves such as *Olea europea*, *Cercis siliquastrum* and *Ligustrum vulgare*. Nevertheless, the typical association between dorsiventrality and hypostomy was observed in all the species possessing the less inclined leaves (e.g. *Citrus aurantium*, *Pittosporum tobira*, *Grevillea robusta*, *Jacaranda mimosifolia* & *Ficus retusa*). On the other hand, among a total of 48 species examined, the exclusive restriction of stomata to the abaxial epidermis grooves was observed in only one xerophytic species, i.e. *Nerium oleander*. In accordance with Burrows' report (2001), sunken stomata were observed in species of the genera *Acacia* and *Eucalyptus*. Of the total number of species studied, 28 % possessed simple (23 %) or compound (77 %) trichomes in abaxial and/or adaxial leaf side(s). The presence of grooves and trichomes is well known to be a plant structural adaptation to sunny and warm habitats, avoiding plant dehydration trough the limitation of transpiration.

Ecophysiological traits of sun and shade leaves. The level of incident sunlight may have strong effect not only on orientational properties of the leaves but also on their structural properties. The most important effect of the amount of incident sunlight on leaf structural properties is the development of sun- and shade-type leaves (e.g. Smith & Nobel 1978). This plant response to the level of sunlight exposure is considered as tolerance adaptation (Smith & al. 1998). But in this case, following their specific location on the canopy, and the concomitant available incident light level, the leaves of the same individual tree present adaptive modifications of both structural and

Figure 6.
Relative occurrence of tree species with similar leaves or sun and shade leaves among 48 broad-lamina leaf species present at Fez city.



functional nature (Hansen 1917; Walter 1973). These intra-individual variations in leaf traits are reflecting a phenotypic plasticity. Within this study, the presence of sun and shade leaves was observed in 29 of the 48 examined broad lamina leaf species (fig. 6). The portions of species that had these two structural leaf-types represented 83.4 % and 28.6 % of the exotic and native examined species, respectively (data not shown).

It is noteworthy that the majority of species that showed the most marked leaf orientational response to high light exposure presented also tolerance response consisting in the development of sun and shade leaves. Thus, the two leaf-types were present in six of the seven observed species with vertical leaf display (*Eucalyptus citriodora*, *E. gomphocephala*, *Acacia cyanophylla*, *Salix babylonica*, *Schinus molle* and *Brachychiton populneus*); the only one exception was the species *Celtis australis*. Generally, shade leaves have greater size (leaf area) than sun leaves (Thompson & al. 1992; Reich & al. 1998). Leaf size directly affects light interception, light penetration through the canopy and leaf energy balance (Ackerly & Reich 1999). The variation in leaf area is determined by three main components: biomass allocation to the leaf, leaf thickness and leaf tissue-mass density, i.e. leaf tissue volume produced by a given amount of dry matter (Dijkstra 1989; Ryser & Lambers 1995). The area increase in shade leaves represents a phenotypic response to the low level of available irradiance, leading to the improvement of aboveground resources acquisition (Lambers & Poorter 1992). Among the 48 species sampled, a broad range of leaf sizes was observed, varying from 5.9 cm^2 in *Myrtus communis* to 1131.8 cm^2 in *Ailanthus altissima*, with 101.2 ± 17.8 cm^2 as mean (± SEM) value (data not shown). In all the species possessing both leaf-types, leaf size was significantly greater in shade leaves than in sun leaves, with mean values (± SEM) of 112.9 ± 22.1 cm^2 and 58.1 ± 12.6 cm^2, respectively (fig. 7A). The leaf area increase in shade leaves, compared to sun leaves, was variable from 15.1 % in *Salix babylonica* to 305.9 % in *Populus alba* with a mean value (± SEM) of 117.9 ± 14.4 %. Higher leaf area in shade leaves, tends to maximise light capture; consequently, at the whole tree scale, the larger total leaf area in the less sun-exposed part of the canopy may result in a compensation of photosynthesis in lower irradiance levels. In sun leaves, a smaller leaf dimension may result in functional advantages in the sunnier, warmer exposed canopy, leading to lower leaf temperatures via increased convective heat loss, as well as substantially lower levels of captured light, avoiding photosynthesis decrease through photo-inhibition (Osmond 1994; Smith & al. 1998). In general, leaves are often smaller in species occupying habitats with high light (see references in Ackerly & Reich 1999). In accordance with this, the species that showed a light avoidance habit, through vertical leaf display, had a relatively small leaf size (data not shown); thus, the mean leaf area value of these species (25.52 ± 3.06 cm^2) was significantly lower than the mean value of the 48 species sampled (101.2 ±

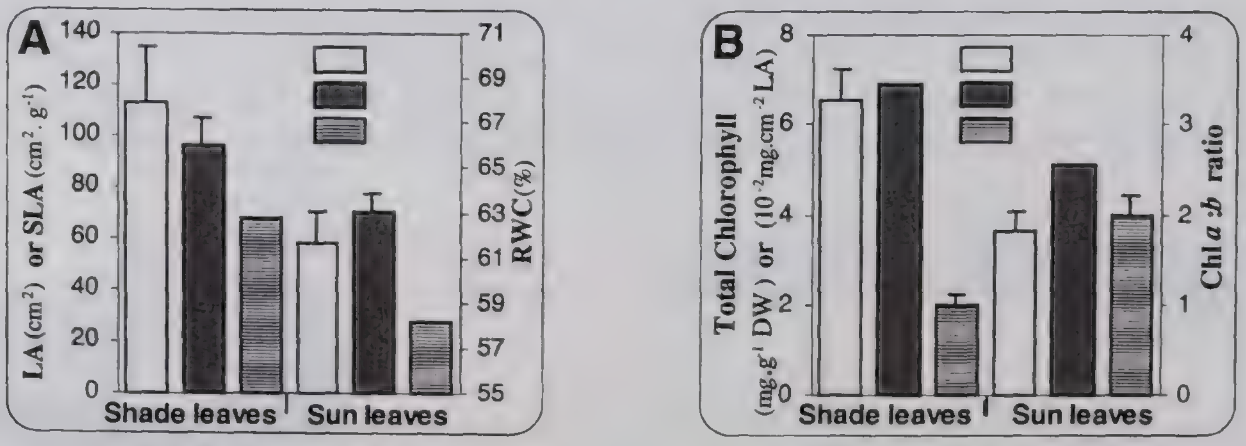


Figure 7. Comparison of sun and shade leaves in respect of some of their ecophysiological traits: A, leaf area (LA), specific leaf area (SLA), leaf relative water content (RWC); B, DW-based and leaf area-based total chlorophyll content, Chl a:b *ratio. Values represent mean ± standard error of the mean (SEM) of data measured in 48 broad-lamina leaf tree species sampled among the trees of Fez city.*

17.8 cm^2). Nevertheless, leaf size also exhibits considerable variation among ecologically similar species within the same habitats, partly in association with canopy architecture and branching morphology and to a lesser extent to reproductive morphology (Ackerly & Donoghue 1998). Thus, the relationship between leaf size and function is not always completely explained, in terms of plant response to abiotic conditions (Givnish 1987). In contrast, specific leaf area (SLA), which represents a main subdivision component of the relative growth rate (Hunt & Cornelissen 1997; Ryser & Eek 2000), is more tightly related to several leaf functional parameters, i.e. assimilation rate, nitrogen content, leaf life span (Ackerly & Reich 1999). Furthermore, the SLA value may reflects leaf thickness, leaf tissue-mass density or both. In the group of 48 species studied, SLA was greatly variable; increasing from 22.2 $cm^2.g^{-1}$ in leaves of *Myrtus communis* to 282.5 $cm^2.g^{-1}$ in shade leaves of *Phytolacca dioica* (data not shown). In this species, SLA was significantly lower in sun leaves (188.3 $cm^2.g^{-1}$) than in shade leaves (282.5 $cm^2.g^{-1}$). Similarly, for all the sampled species, values of SLA were significantly lower in sun leaves than in shade leaves, the respective mean values (± SEM) were 70.8 ± 7.0 $cm^2.g^{-1}$ and 96.2 ± 10.1 $cm^2.g^{-1}$ (data not shown). In general, these SLA values were lower than those obtained by Hunt & Cornelissen (1997) on 16 temperate woody species (trees, shrubs and one climber). In that study, the mean SLA value was 286.9 $cm^2.g^{-1}$, varying from 155.8 $cm^2.g^{-1}$ in *Ilex aquifolium* to 426.2 $cm^2.g^{-1}$ in *Rhamnus catharica.* In species common to both studies, the values reported by Hunt & Cornelissen (1997) were also greater than those determined in the present study (data not shown). Thus, in *Ligustrum vulgare*, *Aesculus hippocastanum* and *Malus sylvestris*, SLA values ($cm^2.g^{-1}$) reported by Hunt & Cornelissen (1997) were 191.4, 233.4, and 236.4, respectively; whereas the respective values obtained in this study were in shade/sun leaves 73.7/51.3, 130.4/121.0 and 105.9/70.6. The difference between the results of both studies is probably related to the fact that the plants used by Hunt & Cornelissen (1997) were largely youngest (21 days) and less light-exposed (PAR: 135 $\mu mol.m^{-2}$) than the mature trees sampled in the present study. Otherwise, fig. 8 shows that among the 48 species examined, SLA mean value was approximately twofold in short-lived leaves (low lifespan) of deciduous species (108.0 ± 2.0 $cm^2.g^{-1}$) than in long-lived leaves (high lifespan) of evergreen species (57.7 ± 1.0 $cm^2.g^{-1}$). In general, the differences in SLA values of sun vs. shade leaves or evergreen vs. deciduous presented here are in agreement with other prior studies (Walters & Field 1987; Abrams & Kubiske 1990; Hunt & Cornelissen 1997; Ackerly & Reich 1999; Ryser & Eek 2000). The higher SLA is considered to be a result of thinner leaves and/or lower leaf tissue-mass density (Dijkstra 1989; Garnier & Laurent 1994; Mulkey & al. 1995; Reich & al. 1999; Ryser & Eek 2000).

The strong negative relationship between SLA and leaf lifespan in deciduous vs. evergreen species suggests that structural reinforcement of leaves plays a major role in determining their lifespan, presumably by rendering them less susceptible to environmental hazards. One of the two components of SLA, i.e. leaf tissue-mass density (dry mass : fresh mass ratio), is negatively correlated to leaf relative water content (percentage of fresh mass minus dry mass to fresh mass). The later parameter was determined for the 48 species sampled; average value was slightly greater in shade leaves (63.01 ± 0.01 %) than in sun leaves (58.00 ± 0.02 %). Minimal and maximal increases, in shade leaves, were observed in *Phytolacca dioica* (0.25 %) and *Cydonia vulgaris* (29.7 %), respectively (data not shown). Thus, these results confirm that the observed higher values of SLA in shade leaves were concomitant to lower values of tissue-mass density. Otherwise, shade leaves of all the surveyed species contained more DW- and leaf area- based total chlorophyll (mean values ± SEM of 6.550 ± 0.710 $mg.g^{-1}$ and 0.069 ± 0.002 $mg.cm^2$, respectively) than sun leaves (3.640 ± 0.410 $mg.g^{-1}$ and 0.051 ± 0.002 $mg.cm^2$) (fig. 7A). The increase of leaf area-based total chlorophyll in shade leaves, compared to sun leaves, varied from 4.3 % in *Brachychiton populneus* to 83.3 % in *Salix babylonica,* with a mean (± SEM) value of 36.8 ± 6.8 %. In contrast, the mean value (± SEM) of DW-based total chlorophyll increase in shade leaves was of 92.3 ± 17.1 %, whereas minimal and maximal increases were observed in other species, i.e.

Aesculus hippocastanum (23.9 %) and *Prunus armeniaca* (221.5 %), respectively. Such influence of light level on the total chlorophyll content of shaded or sunlit leaves was reported in other species elsewhere (Cavender-Bares & al. 2000). The comparison between mean values (± SEM) of leaf area-based total chlorophyll in 22 dorsiventral (0.067 ± 0.03 mg.cm^{-2}) and 7 isobilateral (0.064 ± 0.017 mg.cm^{-2}) sampled species, did not reveal a significant difference (data not shown). Thus, the development of palisade layers beneath both leaf sides, in isobilateral species, appeared accompanied by a partitioning of the chlorophyll amount between adaxial and abaxial palisade tissues, rather than a net increase in total chlorophyll content of a given leaf area. Average leaf chlorophyll *a*:*b* ratio (± SEM) was twofold in sun leaves (2.01 ± 0.14) than in shade leaves (0.99 ± 0.22) of the species examined (fig. 7B). The chlorophyll *a*:*b* ratio decrease in shade leaves, compared to sun leaves, presented a mean value of 392.5 ± 64.5 %; minimal and maximal values being 1.5 % and 1658.1 %, in *Citrus aurantium* and *Cydonia vulgaris,* respectively. Such reduction in leaf chlorophyll *a*:*b* ratio, concomitant to shade acclimation, already reported in many plant species (Anderson & al. 1988; Hidema & al. 1991; Mae & al. 1993), has been partly attributed to the conversion of a portion of chlorophyll *a* to *b* during leaf development in shade (Thomas 1997). Consequently, leaves developing in shade had a higher ratio of light harvesting (relatively chlorophyll b-rich) to reaction centre (a-rich) complexes than those exposed to full sun light, which represent another functional adaptive modification enabling more light capture in shaded conditions.

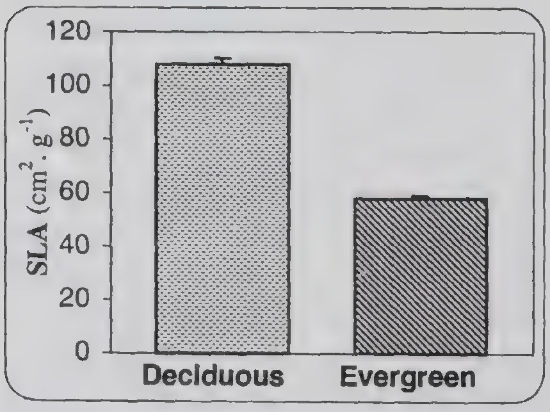


Figure 8. Variation of SLA between leaves of deciduous and evergreen tree species. Values represent mean ± standard error of the mean (SEM) of data measured in 48 broad-lamina leaf tree species sampled among the trees of Fez city.

Summary and conclusions

The management of urban environment requires a good knowledge of its different components. The perennial vegetation is one of the most important components that need to be studied with multidisciplinary approaches.

In this context, the present communication summarizes the results of several multidisciplinary studies carried out on the trees of Fez, a millennial city of Morocco. The listed species are mainly angiosperms, equally distributed between deciduous and evergreen guilds, belonging to 40 families, dominated by Leguminosæ, Rosaceæ, Moraceæ, Palmæ and Rutaceæ. Although most of the species are exotic, they are well adapted to the semiarid climate of Fez. Leaf morphological, physiological and ecological traits were examined as indicators of the trees' adaptive strategies to different environmental constraints. Thus, within the tree species recorded in Fez, a truly striking diversity of leaf form was observed, from simple to compound and from entire-margined to highly lobed. But, more than half of the species had large blade, entire-margined, simple leaves. A considerable variation was also observed in other leaf features reflecting particularly structural and functional responses to the sun-exposure level. Thus, leaf bicoloration was observed in 30 % of Fez tree species; it was mainly shared by species possessing simple leaves characterised by hypostomy, dorsiventrality and horizontal display features, which are generally considered as leaf coordinated responses to shaded conditions in the original natural habitats. Also, sun-avoidance response was observed in some species, reflected by a strong leaf inclination. Vertical leaves, were significantly smaller than horizontal, and occurred mainly in species with both isobilateral and amphistomatous anatomical features, corresponding to adaptive modifications optimising photosynthetic activity in sunny (and warm) habitats. In addition to these inter-specific variations in the leaves response to light levels, intra-individual variations were also observed in species characterised by phenotypic plasticity. Thus, more than half of 48 species examined, possessed two leaf-types, i.e. sun and shade leaves, reflecting different structural and functional acclimation responses to the uneven amounts of sunlight striking different parts of the canopy. As a phenotypic response to shade conditions, shade leaves had significantly higher values of leaf area, specific leaf area (SLA), relative water content, total chlorophyll concentration and chlorophyll *b*:*a* ratio. These leaf traits, generally associated with thinness, low tissue-mass density, great light harvesting to reaction centre complexes ratio, hypostomy and dorsiventrality tend to maximise light capture in low irradiance conditions. Conversely, the sun leaves' structural and functional traits co-participate to the avoidance of photosynthesis photoinhibition and excessive leaf temperature in the full sun-exposed canopy. Thus, the structural and functional traits of both leaf-types, reflecting phenotypic adaptive responses to abiotic constraints, tend to optimise the acquisition of the most important aboveground resources, i.e. light and CO_2. On the whole, this study highlights both floristic and ecophysiological diversity of the tree species of Fez City. Such study could be interesting for not only scientific knowledge, landscaping, urban planning and plant heritage conservation but also for educational purposes. Botanical and environmental educations as well as conservation are the main tasks of Fez Botanic Garden, which is in the process of being established with joint efforts of local scientists, town council members and NGO, but which requires also an additional international scientific and/or financial collaboration.

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The Global Biodiversity Information Facility (GBIF) and the German response

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Abstract. *– The Global Biodiversity Information Facility was founded in 2001, with the principal objective of making Biodiversity data freely and universally available. GBIF consists of countries and organisations (participants) that have signed a Memorandum of Understanding. The dissemination of biodiversity data is organised by Participant Nodes. The Austrian and the German National Nodes represent two models for national Node organisation. The Austrian Node is centralised at one institution while Germany has set up a system of seven Nodes to organise her contribution. The German Botanical Node is further described, in which botanical gardens play an important role as the showcase for biodiversity.*

Key words: *biodiversity, GBIF-D, Memorandum of Understanding, National Node, participation, botanical garden, collection, herbarium, database, network*

Introduction

The Global Biodiversity Information Facility (GBIF) is an international initiative with the prime aim of providing free and universal access to the world's biodiversity data. The term "biodiversity data", as used in this context, embraces specimen information from conserved and living collections and observation records as well as taxonomic information and descriptive data. The signatories to the GBIF Memorandum of Understanding have each agreed to form one or more "Participant Nodes", which are responsible for the organisation of their network. Participants are autonomous in choosing a way of actually setting up their own network, as exemplified by the Austrian and German GBIF Nodes.

This paper gives an introduction to the aims and the structure of that initiative, with a focus on the decentralised organisational structure of the German GBIF Node system. The German botanical Node and the role of botanical gardens within GBIF will be discussed in more detail. Future perspectives of GBIF are briefly explained.

GBIF International

Biodiversity and biodiversity data. The term biodiversity – or biological diversity – covers the variety of life on earth as well as its temporal and spatial patterns. The Convention on Biological Diversity (CBD) was adopted by the vast majority of the world's governments at the Earth Summit in Rio, 1992. The preservation of biological diversity, the sustainable use of its components, and fair and equitable

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sharing of the benefits from the use of genetic resources have been formally identified as the main goals of the CBD (CBD 2000).

The value of biological diversity has often been discussed and demonstrated. About 1.75 million species have been identified, and estimates about the real numbers range from 3 to 100 million (CBD 2000). One of the major challenges remains the task of increasing our capacity to document and understand biodiversity, and to assess more accurately its value, in order to understand the threats to it. In this, collections and observation records play a major role, being the archives of past biodiversity that allow a reconstruction of development over time. However, up to day, the biodiversity related data and object collections are largely accumulated in countries of the so-called developed world, while the hot spots of life biodiversity are located in the developing world.

Biodiversity data at the level of individual organisms are held in natural history collections, living organism collections, herbaria, and as observation data. They can be found in databases, in card file systems, and in libraries. Often the data consist only of the specimens themselves, but increasingly they are transferred to electronic form. Though exchange standards do exist and are being developed (Berendsohn 2003, 2003a), uniform approaches to structuring biodiversity data are rarely applied in databases, which makes the compilation of data very time-consuming if not downright impossible.

The exchange of biodiversity information is further hindered by the fact that the access to information on organisms is mainly by name – and names can be misapplied, can follow different taxonomic concepts, or just represent an erroneous determination. This is challenging for everybody who tries to draw together factual information on a given taxon from different sources, though several approaches now exist to record more accurate information on the application of names (Koperski & al. 2000, Geoffroy & Berendsohn 2003).

What is GBIF? The central aim of the Global Biodiversity Information Facility (GBIF) is to make the world's biodiversity data freely and universally available. GBIF came into existence as the result of the Working Group on Biological Informatics of the OECD Megascience Forum, a body focused on large-scale international scientific collaboration. The Biodiversity Informatics subgroup of the Working Group recommended the establishment of an international co-ordinating body and a new data network called the Global Biodiversity Information Facility (GBIF).

The Working Group identified a need in science as well as society for biodiversity information. The business plan of GBIF (2002) states: "A major scientific consideration in biodiversity science is the need to bring 250 years worth of accumulated information in an electronically available format." GBIF co-operates with several other international organisations concerned with biodiversity. These include the Clearing House Mechanism (CHM) and the Global Taxonomic Initiative of the Convention on Biological Diversity, as well as regional biodiversity information networks. The Memorandum of Understanding (MoU) that GBIF is based on provides the guidelines for participation, work plans, intellectual property rights, and others. The OECD science ministers endorsed GBIF in 2000 and GBIF came into existence in March 2001, when ten countries had signed the MoU. The initial term of GBIF is five years. Meanwhile (as of October 2003), 39 countries and 22 organisations participate in GBIF.

GBIF is evolving to be an interoperable network of biodiversity databases and information technology tools. The biodiversity information brought together by connecting data sources to this network will be freely available, i. e. published on the Internet free of charge.

The most important principles of GBIF concern first of all a close coordination in order to deploy common standards and to avoid a duplication of efforts, and secondly leaving the data providers with full control over their data, while the data are shared using a distributed network architecture. In accordance with the CBD, by providing open access to biodiversity data GBIF will help to level out the inequality of data distribution, so that developing countries will have the means to retrieve and use the data that are relevant to their own biological diversity.

Organisational structures. Participation is primarily open to countries and organisations. Signing the Memorandum of Understanding is the necessary basis for participation. With signing this Memorandum, the participants agree that they are willing to promote the sharing of biodiversity data in GBIF. There are basically two ways of participation: as a voting participant, or as an associate participant. Voting participants make a financial contribution to GBIF, and have a vote in elections of the Governing board. Mainly for organisational reasons (to avoid complications in voting procedures due to overlapping memberships), only countries can be voting participants. Associate participants may be countries as well as economies, like the European Union, or Non Governmental Organisations like Species 2000 or IUCN (The World Conservation Union). They take part in the deliberations of the Governing Board, but may not vote, and they make no direct financial contribution to GBIF.

The Governing Board consists of the representatives of the participants who have signed the MoU. Voting participants (i.e. those that have paid a contribution) elect the officers of the Governing Board: the chair of the Board, of the Budged Committee, of the Executive Committee, of the Participant Node Managers Committee, of the Science Committee and of its Subcommittees. The secretariat of GBIF with its scientific and administrative staff is hosted by the Zoological Museum of the University of Copenhagen in Denmark.

In signing the MoU, participants agree to "form a node or nodes, accessible via GBIF, that will provide access to biodiversity data." (MoU 2000). A Node is here defined as being "a stable computing gateway that allows real-time inter-operational search on databases containing primary or meta-level biodiversity data, or a single, web-accessible computer containing one or more significant maintained biodiversity databases." The basic idea of GBIF is to set up a network of Nodes in which the responsibility for the content is left with the content producers. Nodes providing content (biodiversity data or software tools for processing biodiversity data) are called "Data Nodes". A "Participant Node" in GBIF terminology is the officially named representative of a participant, who is responsible for the organisation of the part of the network the participant brings into GBIF. Participant Nodes control the registration of Data Nodes for GBIF, and they are themselves often also Data Nodes. The Participant Nodes Managers Committee (NODES) of the Governing Board attempts to coordinate the Participant Nodes' efforts.

The Work Programmes. In fulfilling the task of providing biodiversity data to the network, the participants are supported by the GBIF secretariat. The four Work Programmes of GBIF are to address issues that concern the GBIF network as a whole and that would result in a duplication of effort if tackled individually by participants.

The **DIGIT** (Digitisation of Natural History Collections) work programme concentrates on finding cost-effective ways of making digitisation efforts easier and of speeding up the process of data mobilisation. This task includes the development of a strategic approach to tackle the digitisation of about 1.5 billion specimens (L. Speers, pers. comm.), the mobilisation of an unknown number of observation records, defining priorities to optimise the use of resources, and last but not least providing useful results in time for the first evaluation of GBIF (to take place in 2004).

The **ECAT** (Electronic Catalogue of Names of Known Organisms) work programme focuses on names assigned to taxa. Its aim is to establish access to a reliable source of names, synonymies and classifications on the Internet. As names are the key handles to organism related information, ECAT forms a central component of the GBIF network. It will be set up in cooperation with existing initiatives such as Species 2000 and ITIS (Integrated Taxonomic Information System) , which have already started to provide unified access to name-related databases. In a network of independent sources, however, drawing together meaningful information will strongly depend on there being a sound base of nomenclatural data and on a system that supports conceptual relationships between named taxa.

While DIGIT and ECAT focus on the data providers' side to mobilise content, **DADI** (Data Access and Data Interoperability) is dedicated to building the infrastructure for the Global Facility. DADI is concerned with the technical design of network components such as communication and query protocols, indices, central registry of data sources, etc. It also drives the development and implementation of data standards, an essential prerequisite for the integration of information within the GBIF network. The Secretariat's Information and Communication Technologies (ICT) staff supports DADI by providing software, documentation, and a helpdesk.

The activities of the **OCB** (Outreach and Capacity Building) work programme cut across those of the other work areas. It organises technical training workshops, helps to set up new Participant Nodes, promotes the further participation in GBIF, and attempts to increase the number of people who benefit from the use of GBIF. OCB specifically focuses on furthering the involvement of developing countries.

Each of the work programmes is supported by a programme officer at the secretariat and by a subcommittee of the Governing Board's science committee, which consists of independent experts.

National Nodes

In signing the MoU, participants in GBIF declare their commitment to provide access points to their biodiversity data. Managers of the National Nodes within participant countries act as the "local organisers" of their respective national part of the global network. Countries are completely autonomous in deciding on the way in which to organise their own National Nodes, as long as they provide a GBIF-compliant access to the data sources. This results in a number of different approaches, depending on what suits a participant best in terms of technical as well as human infrastructure and resources. Before focussing on the German approach to organising National Nodes for GBIF, the example of Austria will be briefly introduced.

GBIF Austria. The National Node of Austria is located and organised at one point, the Federal Environmental Agency in Vienna. Austria began with a survey of all known private and public collections and databases. This survey identified a total of 498 collections in Austria, including both public an private reference and exhibition collections. The majority of them is used for research, conservation and teaching. These collections contain more than 46.5 million objects. 113 databases with a total of about 8 million records have been identified. The survey has been summarised in a report (Götzl & al. 2003), which also gives guidelines for the further development of the National Node. Initially, the databases that already exist will be connected to GBIF. The report expects that six databases will be accessible after one year and 85 databases after a period of five years. The system will most likely be a mixture of central data storage in a computer centre, and distributed data storage of individual databases accessible via a network interface. Presently, information about Austrian biodiversity collections is retrievable from a meta-database. This database is accessible over the Internet (http://www.biodiv.at/gbif/index.htm).

GBIF Germany. In contrast to the Austrian example where organisation is centralised, the German National Node is actually composed of seven Participant Nodes, which cover different taxonomic groups (see http://www.gbif.de).

This structure was decided upon during several preparatory meetings (see http://www.gbif.de/gbif-de/Geschichte/index_html) organised by the German Federal Ministry of Education and Research, which is also providing the start-up funding for the projects centred at the Nodes. This structure was thought to be more useful than a strongly centralised one because it can more effectively reach out to the research community, using existing institutional and personal connections. Scientists and thus the primary data providers tend to be more comfortable with the effort led by an institute that is a recognised centre of research. Furthermore, the existing organisation of data provision vary between the groups, and thus the priorities chosen for the start-up projects may vary considerably. The coordinating institutes were chosen by workshops of the respective communities. The following Nodes were formed:

- **Prokaryotes and Viruses**: German collection of Microorganisms and Cell Cultures, Braunschweig (DMSZ)
- **Mycology**: Botanische Staatssammlung, München (Botanical State Collection, Munich)
- **Botany**: Botanic Garden and Botanical Museum, Berlin-Dahlem (BGBM)
- **Evertebrata I** (Insecta): Staatliches Museum für Naturkunde, Stuttgart (State Natural History Museum, Stuttgart)
- **Evertebrata II** (Mollusca, Chelicerata, Myriapoda): Zoologische Staatssammlung, München (Zoological State Collection, Munich)
- **Evertebrata III** (Cnidaria, Annellida, Crustacea, Bryozoa): Forschungsinstitut Senckenberg, Frankfurt / M. (Senckenberg Natural History Museum, Frankfurt / M.)
- **Vertebrata**: Zoologisches Forschungsinstitut und Museum König, Bonn (Zoological Research Institute and Museum König, Bonn)

The Federal Ministry of Education and Research supports the setting up of the German GBIF Node system with a total of 6.6 Mio Euro for a period of three years. The Nodes have each proposed and organised an umbrella project; in all, 39 institutes carry out 58 sub projects.

The projects of the Nodes focus on different aspects of data provision and organisation of the GBIF network. The zoological Nodes will primarily build a digital catalogue of primary type specimens in German natural history collections. Three of them will form a common Data Node, using the SysTax System at the University of Ulm as their data warehouse.

The Node Evertebrata III uses the collection management system SeSam, developed and located at the Senckenberg Institute in Frankfurt / M. The microbiological Node will focus on common data provision of strain collections in a number of institutes. It is now building the information infrastructure at the DSMZ (German Collection of Microorganisms and Cell Cultures) in Braunschweig, and probably it will also form a centralised database and Data Node. The mycological and botanical Nodes support decentralised solutions for data input and maintenance. The approach of the botanical Node is described in more detail below. The Mycology Node is a network of databases using a common software, the DiversityCampus (http://160.45.63.11/menu) suite of software tools.

In addition to the projects at the Nodes, an additional grant covers cross-cutting issues, such as the organisation of the information flow within the Node system, workshops for the IT specialists involved, the planning for long-term provision of the system, a helpdesk, and the connection of collection Data Nodes to the global system. This will be accomplished using the software system provided by the EU-financed BioCASE project (www.biocase.org).

The German Botanical Node

Of the seven German GBIF Nodes, the botanical Node (see http://www.gbif.de/botanik) is the most complex. This is principally due to the existence of several networks already providing data relevant to GBIF. These systems must be properly coordinated and included, avoiding duplication of efforts and giving full recognition to all stakeholders involved. Another important reason for the complexity of the task is the sheer number of institutions involved. A survey of collections revealed 117 institutions holding living plant collections (most of them botanical gardens) and 75 herbaria (Klingenstein & al. in press). From the start, a conscious effort has been made to include all biodiversity-related botanical sub-disciplines (taxonomy, surveys, natural history collections, culture collections, in-situ and ex-situ preservation), and to reach a broad coverage of the taxonomical groups within the scope of the botanical

Node (protists, algae, mosses, and vascular plants). In the current project phase, the botanical Node coordinates ten subprojects. Some of them concentrate on the digitisation of data (mostly specimen data and images), while others have their main focus on the linking of existing databases. Existing databases contain data referring to taxonomy, vegetation, botanical garden accessions, protection status of species, as well as cultivated plants and plant genetic resources.

As a structural framework for the organisation of the Node, work fields were divided into "areas of competence", so as to make use of existing infrastructure, community efforts and expertise. This is not only in order to include data sources from all these areas, but it is also seen as a prerequisite to set up a lasting network beyond the phase of initial project funding.

In the area of **taxonomy**, checklists and floras of German plants will be collated. These lists contribute to GBIF's catalogue of names and form the taxonomic backbone for access to plants in Germany. **Plant genetic resources** include collections and databases referred to cultivated plants like crop plants and forest resources, which are stored at the Institute of Plant genetic Resources (IPK) in Gatersleben and at the Information Centre for Biological Diversity (IBV) in Bonn. **In-situ phytodiversity** principally concerns the data stemming from decades of floristic mapping in Germany. These data are collated and accessible via the Portal 'Floraweb' of the Federal Agency for Nature Protection (BfN) in Bonn, where data of distribution, ecology, red lists, vegetation ecotypes and vegetation maps of German plants are retrievable. This gateway will in the future also provide data on the ecological traits of plants in Germany. **Botanical gardens** have a long tradition in material exchange, data standardisation and data storage, providing information on the provenience of plants and often of their cultivation history. They are popular and are probably the only botanical collections that are noted by the general public. Botanical gardens are a display case of biodiversity. They have a high potential of forming the GBIF connection point to non-scientists in society. Data collation for German Botanical Gardens is organised by the Verband Botanischer Gärten (Association of Botanical gardens in German speaking countries), using the SysTax database at the University of Ulm. For German **herbaria** a central access portal will result in the German Virtual Herbarium.

All these databases and systems will be connected and therefore accessible via the Botany Node of the German GBIF system. Data from multiple sources and different research domains can then be combined in novel ways to derive information not existing in any single data source. For example, data of specimens will be connected to observation data. Thus a comparison of historical distribution data - stored in herbaria - with current distribution information of in-situ phytodiversity observation records is possible. This could, e.g., further facilitate the tracking of invasive species.

Perspectives

The international GBIF project represents an enormous opportunity but also a challenge for biological institutes and researchers, particularly those concerned with systematics and systematics collections. If GBIF succeeds to provide access to global biodiversity information, it will open a wide range of new research opportunities thus allowing us to help stem the global loss of biodiversity. It will also provide additional proof for the utility of our research and collections, which we can use to ensure their maintenance. Success will depend on the rapid mobilisation of existing information, for which the cooperation of all providers of primary biodiversity information is required.

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The Biodiversity Conservation Centre of Cagliari (Italy)

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Abstract. – *The Biodiversity Conservation Centre of Cagliari was established to study, manage and conserve the plant diversity in Sardinia. The strategy followed by the centre is to preserve the highest number of Sardinian endemic taxa and those having phytogeographical interest, both as seeds or spores, and as cultivated plants for* ex situ *conservation. Moreover, the Centre aims to study taxonomy, biosystematics and ecology of species in danger of extinction, and to propose the most fitting strategies to save the habitats where they live. Two projects have been started in the last two years: one to preserve threatened species in the province of Cagliari, the other to study the plant diversity in the west Mediterranean insular area. At present, the germplasm of more than 200 taxa is cryopreserved, while 250 entities have been multiplied in pots and in the rock garden. At the same time, floristic and biosystematic studies resulted in description of novelties in taxonomic revisions, determination of chromosome numbers and publication of local floras. The draft of a law regulating the protection of Sardinian flora has been submitted to the Regional Council.*

Key words*: biodiversity, conservation, seed bank, Sardinia.*

Introduction

The Biodiversity Conservation Centre of Cagliari (CCB) is a structure of the Botanical Department, University of Cagliari, established to study, manage and preserve the plant diversity in Sardinia. CCB was officially settled in April 2003 by a resolution of the Department board. Its foundation was possible thanks to MIUR (Ministero Istruzione, Università e Ricerca) funding (Law number 6-10.1.2000), which enabled the purchase of scientific instruments and machinery. The CCB includes a seed bank and a biodiversity rock garden, lately completed, and publishes an Index Seminum.

Aim of the centre is to preserve the highest number of Sardinian endemic taxa possible as well as those having phytogeographical interest, both as seeds or spores, and as cultivated plants for *ex situ* conservation. Moreover, the CCB aims to study taxonomy, biosystematics and ecology of the threatened species, and to propose the best strategies to preserve their habitats (Bocchieri & al. 2000).

Concerning the collaboration with other institutions, there are many projects of research and scientific popularization, e.g. two of them started in the last two years: a first one to preserve the species in danger of extinction in the province of Cagliari (Bocchieri & al. op.cit.), and a second one to study plant diversity in the W-Mediterranean insular area (Bacchetta & al. 2001a). These

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initiatives have been accomplished in collaboration with the Provincial Administration of Cagliari and the Jardì Botanìc of the University of Valencia (Spain).

CCB structures

CCB includes a seed bank, two research laboratories, six experimental fields and the rock garden for *ex situ* conservation; it publishes an Index Seminum. Though the seed Bank project dates back to 1997, places for its development were chosen only after an agreement with the local administration of Cagliari. Subsequently, all the buildings were completed and equipped with machinery and cold stores. In particular, two rooms for the processing of germplasm, one room for cold stores and the seed bank were built, together with a greenhouse with thermo-regulated benches for the study and propagation of the plant material. At present, the bank preserves the germplasm of more than 200 taxa belonging to the Sardinian Flora.

The preservation of all the most representative species of the W-Mediterranean insular area is going to be achieved in the next future, as planned in the above-mentioned Cagliari-Valencia project.

The Index Seminum is the oldest publication of Botanical Department: the first has been published by Patrizio Gennari in 1885. Since then the publication went on with only few forced interruptions until the year 2000. During the last years, in addition to the species collected in the Botanic Garden, species growing in Isola dei Cavoli (Villasimius, Cagliari) were also included. Today, after a pause of three years, due to economical and technical reasons, the Index ceased to be printed and became available on-line in a digital form. The germplasm in the Index Seminum has also changed, according to the biodiversity conservation policies adopted by the CCB. As a matter of fact, over the last years, several different strategies have been devoted to the conservation of endemic or phytogeographically interesting species existing in Sardinia and nearby territories. Two labs were appointed to conduct biosystematic researchs and to process and keep germplasm. At the moment, the staff operating in these structures includes one researcher (Gianluigi Bacchetta), three technicians (Paolo Atzeri, Marco Pitzalis, Roberto Sarigu), three researchers under contract (Mauro Casti, Gianluca Iiriti, Cristiano Pontecorvo) and one fellowship INTIME 36 (Alessandro Demurtas). Moreover, the structure works in collaboration with one Spanish fellowship (Pedro Català), and five students who are working on their degree thesis at the CCB: Martino Orrù, Laura Piras, Riccardo Vacca from the University of Cagliari; Laura Zavattero from the University of Rome "La Sapienza"; Francesca Manconi from the University of Pisa.

The experimental fields consist of a terraced area divided in three sectors, and three further fields where the multiplication of seeds occurs. The terraced area is used for the cultivation of biosystematically investigated taxa; the other fields are assigned to the growth of specimens for a future conservation *in situ*. The rock garden for biodiversity is devoted to *ex situ* conservation and it is also used for didactic and popularization activities. Before, the Botanic Garden had only few small areas intended for that use, but now, since the creation of the rock garden, most of the Sardinian taxa requiring conservation obtained a proper presentation.

The construction of the experimental field started in December 2001, and was completed in March 2003. The area is about 600 square metres, divided into calcareous, granitic and metamorphitic sectors. The installation of propagated and potted species began in Autumn 2002, and is going to be completed in Spring 2004. The project also comprises enlargement by including a nearby rocky area, unused by now due to the steep slope.

Projects

CCB coordinates various research projects, some of which are mentioned in the introduction. It is to be highlighted how the well-established cooperation with the Jardì Botànic of the University of València, together with a strong will to extend similar synergies to other university structures, led to the elaboration of an INTERREG project aiming to the creation of a Mediterranean network of seed banks. This project is called GENMEDOC and it will start over the next two years, during which other Departments and Botanic Gardens of the Mediterranean region (Barcelona, Catania, Chania, Marseille, Murcia, Palma de Mallorca, Tunis, Valencia) will be involved in the development of a common protocol and of a specific software for germplasm conservation, managing and exchange.

The project also intends a thorough study of the themes related to the reproductive biology of those species reported in the E.C. Directive Habitat 92/43 and its updates. The ultimate aim is the creation of a European System for germplasm conservation, in order to prevent the extinction of threatened species and of those having a great phytogeographical interest. Besides projects and collaborations already running between the University of València and Cagliari, a Life project for the conservation of taxa mentioned in the 43/92 Directive was presented together with the WWF and the University of Ancona (Italy). The joining of the IUCN European project for the preservation of biodiversity in the Mediterranean area and the WWF Planet Ecoregion project is expected during the current year. On the side of collaborations with other Italian and foreign institutes, taxonomic and biosystematic researches are currently going on in cooperation with the Universities of Berlin, Catania, Firenze, Trieste, València and Zurich. Other institutes will be involved, among which that of Napoli, with which contacts have been recently established, leading to the study of the herbarium material about Sardinian flora by Nicola Terracciano.

Results

At present it has been possible to cryopreserve the germplasm of more than 200 taxa, and to multiply 250 entities in vase and in the rock garden. At the same time, floristic and biosystematics studies have led to the description of new taxa (Mossa & al. 1999; Bacchetta & Brullo 2000; Bacchetta & al., 2000a; 2003), the revision of taxonomic groups (Mossa & al. 1998; Boscaiu & al. 2001; Bacchetta & al. 2001b), the determination of chromosome numbers (Bacchetta & al. 2000b; Cusma Velari & al. 1999; 2000; 2001; 2002) and the publication of local floras (e.g. Mossa & al. 1996; Mossa & Bacchetta 1998).

Concerning biodiversity, the draft of a law about the protection of Sardinian flora has been presented (Bacchetta & al. 1999); which should be discussed by the Regional council by 2003.

In addition to the already mentioned activities, a series of scientific and popularizing events have been planned to illustrate the results and to sensitize the public opinion to such themes as the preservation of species and habitats.

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Genetic resources conservation – experiences from agricultural research for strengthening collaboration with Botanic Gardens

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Abstract. *– The roles of Botanic Gardens and Agricultural Research Centres (ARC) have evolved with time, creating more overlap. Botanic Gardens are increasingly getting involved in* in situ *conservation and have set up seed banks and other* ex situ *facilities to conserve greater diversity than would be possible in garden collections. ARC on their part have amassed much experience on genetic conservation and can be a very useful source of information for Botanic Garden curators. The different histories and functions of Botanic Gardens and ARC mean that their strengths and areas of expertise are slightly different, which makes them well suited as partners in conservation. This paper focuses on the expertise that ARC can offer to the Botanic Gardens community as a means for strengthening collaboration in plant genetic resources conservation.*

Introduction

Botanic Gardens and Agricultural Research Centres are both facing the global challenge of how to conserve the threatened plant diversity of the planet. Plants are important parts of the food web sustaining life on earth. They provide the essential resources for the livelihood of people in terms of food, medicines, clothing, shelter and fuels (Given 1994; Wilson 1992) thereby contributing to food security and poverty alleviation. In addition plant species and their populations provide an incremental amount of ecosystem goods and services such as water purification, pest control, timber, regulation of biogeochemical cycles, mitigation of floods and droughts (Daily 1997). It is now widely recognised that plant diversity is declining at unprecedented rates (Simberloff 1986; WRI, IUCN & UNEP 1992). There are a combination of factors responsible for this loss of diversity, which include inter alia: habitat loss, pollution, introduction of alien species, climate change and over exploitation (Falk 1987).

Historically both Botanic Gardens and agricultural research centres have reacted to the loss of diversity through deliberate conservation actions aimed at collecting and conserving wild species and crop genepools including their wild relatives, in botanic gardens and genebanks respectively. The roles of these institutions in the conservation of biological diversity is, however, evolving with time and creating more overlap. For instance Botanic Gardens are increasingly getting involved in *in situ* conservation. Many botanic gardens are active in *in situ* conservation maintaining or managing nature reserves, areas of vegetation or working closely with protected areas authorities (Wyse Jackson & Sutherland 2000). In recent years many Botanic Gardens have set up seed banks and other *ex situ* facilities to conserve greater diversity than would be possible in the living plant collections.

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Agricultural Research Centres on their part have amassed much experience on the conservation and management of genetic resources (Scarascia-Mugnozza & Perrino 2002). In the last 30 years, the International Plant Genetic Resources Institute (IPGRI) has been very active in promoting the establishment of genebanks world wide and has been developing materials and technical guidelines for training and supporting the work of genebank curators and staff. The different histories and functions of Botanic Gardens and Agricultural Research Centres mean that their strengths and areas of expertise are slightly different but with specific strategies and action plans their efforts can be complementary (Engels & Engelmann 2002). And this makes them well suited as partners in conservation.

This paper discusses the various aspects of plant genetic resources (PGR) conservation such as exploration and collecting, *in situ* and *ex situ* conservation methods, genebank and germplasm management, germplasm use, training and capacity building, and public awareness and education. It also provides information and support that Agricultural Research Centres, in particular IPGRI can offer to the Botanic Gardens community as a means for strengthening collaboration and to facilitate and promote the exchange of information related to PGR conservation.

Collecting Plant Genetic Resources

Sampling strategy

Collecting plant genetic resources is the first and most critical step for *ex situ* conservation (Guarino & al. 1995) since it will determine how useful and valuable your collection will be. For the genetic resources community, a major preoccupation is to capture the maximum amount of genetic variation in the materials collected. This requires a sound sampling strategy that determines the number of populations and individuals that need to be sampled and collected. There has been a lot of debate about methodologies, amounts and frequencies in sampling plant germplasm (Marshall & Brown 1975; 1981; Namkoong 1988; Brown 1992; Brown & Briggs 1991; Falk 1991; Lawrence 2002; Neil & Cummins 2003). The number of populations necessary to conserve genetic diversity within a species depends on the measure of diversity, how it is partitioned and how much of the total diversity is enough to conserve. It has been determined that sampling from five populations of a rare species would be sufficient to have 90-95% probability of capturing all common alleles for *ex situ* conservation (Brown & Briggs 1991; Falk 1991). Brown & Marshall (1995) recommended that collecting 50 individuals at each of 50 populations could be considered as a benchmark. However, Lawrence (2002) argued that for *ex situ* conservation, a sample of 172 plants drawn from a population of a target species is required to capture all or nearly all of the polymorphic genes that are segregating in a population, and that there is little to be gained by collecting more than a very few seeds from each plant. More recently, Neil & Cummings (2003) assessed the genetic consequences when different number of populations of four plant taxa were collected in terms of how well alleles numbers and expected heterozygosity were represented. Their conclusions were that it is necessary to conserve 53-93% of sampled populations to meet the standard for common alleles (90-95%) and between 20-64% of populations were needed to reliably represent species level heterozygosity.

Besides number of samples, the decision on what to collect is also important. There has also been a kind of paradigm shift from the early days where it was important to quickly collect plant populations, traditional landraces, that were in danger of being replaced by modern varieties to collect germplasm of wild and weedy species of major crops, nationally and locally important crops or to sample or resample populations to fill gaps (Brown & Marshall 1995). It was recognised that for germplasm to be useful it had to meet the needs of breeders for crop improvement programme.

Ecogeographic surveys

In order to make the best decisions, considerable information is required about the nature of the target region (e.g. topography, geology, soil, climate, vegetation etc.) and the target species (distribution, phenology, reproductive biology, genetic diversity, ethnobotany, storage behaviour etc.). Such information can be derived from ecogeographic surveys or studies (Maxted & al. 1995; Maxted & Guarino 1997). Maxted & al. (1995) define an ecogeographic survey as an ecological, geographical and taxonomic information gathering and synthesis process. The results, which are predictive, can be used to assist in the formulation of conservation priorities and deciding appropriate strategies. Vavilov's classic work on the theory of centres of diversity is based on such ecogeographic principles. The spatial analysis of ecogeographic data in the distribution patterns of crops and wild species has been facilitated by the development of geographic information systems (GIS) softwares such as DIVA-GIS (Hijmans & al. 2001a), Floramap (Jones & al. 2001) and Worldmap (Williams 1994). These softwares have been specifically designed for spatial analysis of genetic resources data. The DIVA-GIS software for example has been developed by the International Potato Centre (CIP) in collaboration with IPGRI and support from the System-wide Genetic Resources Programme (SGRP) (Hijmans & al. 2001b). Amongst its many applications in plant genetic resources activities, it has been used in exploring the geographic distribution in wild potatoes species (Hijmans & Spooner 2001) and to guide germplasm collecting of wild *Capsicum* in Paraguay (Guarino pers. com.).

The International Board for Plant Genetic Resources (IBPGR), the precursor of IPGRI, was set up in 1974 with the precise and important task 'to promote and assist in the world wide effort to collect and conserve plant germplasm needed for future research and production' (Scarascia-Mugnozza & Perrino 2002). The collection of germplasm was thus one of IBPGR's main activity in its early years. Such collection was always done in collaboration with National Plant Genetic Resources Programmes as well as other International Agricultural Research Centres (Guarino & al. 1995). By mid 1990's IBPGR had sponsored some 560 missions in about 130 countries for a total of 220,000 samples collected. Approximately 125,000 of these accessions can be found in SINGER, i.e accessions stored in the CGIAR genebanks. Since the early eighties, the number of collecting missions supported by IPGRI has started to decrease, as emphasis shifted from collecting to maintaining collected germplasm and developing capacity within genebanks at national and regional levels. The Convention of Biological Diversity also had a major impact on the number of international collecting missions, as nations became more protective of their biological resources on which they have sovereignty rights.

IPGRI has produced several publications to assist and facilitate partners to undertake collecting missions. The most comprehensive book which deals with collection of plant genetic diversity is the 1995 Technical guideline by Guarino & al. (1995). This book provides detailed guidance on the planning of collection missions, in the field, post collecting and many case studies. More recently Pence & al. (2002) produced a technical bulletin on *in vitro* collecting techniques, which provides valuable information on theoretical background of *in vitro* collecting as well as collecting for wild and endangered species and describes case studies on coffee, cacao, banana, citrus, avocado, taro, coconuts, tropical rainforest trees as well as wild and endangered species.

Conservation methodologies

Ex situ conservation

There are two basic conservation strategies that can be adopted to conserve genetic diversity, namely *in situ* and *ex situ* conservation (Maxted & al. 1997). For *ex situ* conservation, samples of the target taxon are collected and transported to the conservation site, away from its original location. The material can either be conserved as live plants in field gene banks or botanic gardens, or as samples of seeds, tubers, tissue explants, pollen or DNA maintained under controlled conditions in appropriate facilities for seed and *in vitro* storage. Seeds are usually the most efficient and cost-effective form for the storage of plant germplasm. Many crop species have orthodox seeds which can be dried to a low moisture content (3% - 7% seed moisture content, depending on the species) and stored at low temperatures (e.g. -18 °C) without losing their viability over long periods of time, according to international standards (FAO/IPGRI 1994). Indeed, of the about 6 million accessions stored in over 1300 national, regional and international germplasm collections world wide, more than 90% are stored as seeds (FAO 1998).

But not all species produce orthodox seeds and reproduce by seeds. Many plant species, especially of tropical origin, produce recalcitrant or intermediate seeds, i.e. that do not tolerate drying and/or freezing. The number is not as big as those that produce orthodox seeds, but many of them are economically important, e.g. oil palm (*Elaeis guineensis*), rubber (*Hevea brasiliensis*), durian *(Durio zibethinus*), coffee (*Coffea* spp.), cacao (*Theobroma cacao*), coconut (*Cocos nucifera*) and many other tropical fruit trees. Species such as banana (*Musa* spp.), pineapple (*Ananas comosus*), taro (*Colocasia esculenta*) and many other root and tuber crops reproduce mainly through vegetative means such as tubers, root, suckers, crown etc. For all these species, other *ex situ* conservation methods are required, such as field gene banks, *in vitro* slow growth methods or cryopreservation.

In field genebanks, plant genetic resources are kept as live plants. According to FAO (1998), ca. 527,000 accessions are stored world wide in field genebanks. Like any conservation technique, field collection have their advantages and disadvantages. A great advantage of field genebanks is the ready availability of the material for characterization, evaluation, utilization and research. But, just like in the farmer's field, one major constraint is all the natural hazards of farming (pest and diseases, drought, flood, cyclones, etc.). Therefore, field collections should, for safety reasons, be duplicated in another location, or, if possible, germplasm should also be conserved through other complementary methods such as *in vitro* (slow growth) storage or cryopreservation.

The basic aim of *in vitro* methods is to introduce explants (small tissue pieces) from plants into sterile culture and maintain them in a pathogen-free environment. For short- and medium-term conservation, the stored cultures are kept under controlled conditions of slow growth. The frequency of sub-culturing is greatly reduced, thereby prolonging storage and reducing maintenance. Long-term conservation of explants requires storage at ultra-low temperatures, usually -196°C in liquid nitrogen to facilitate the arrest of mitotic and metabolic activities. Individual cryopreservation protocols have to be developed for each species and the basic requirements for cryopreservation are that the plant materials have to survive the dehydration procedure before storage in liquid nitrogen and the thawing procedure after storage, and to be regenerated into whole plants for use (Tao 2001).

Pollen storage is already common practice in breeding programmes to bridge the gap between male and female flowering time and to improve fruit setting in orchards. Pollen used for germplasm conservation should remain viable for many years and cryopreservation of pollen seems to be the most efficient method. For example, maize pollen could be used successfully for pollination after 10 years of cryo storage (Barnabas & Kovacs 1997). In coffee, Walyaro & van der Vossen (1977) have obtained high pollen viability of *C. arabica* after more than two years by storing pollen under vacuum at -18°C.

Also extracted DNA or DNA-rich tissues can be stored in liquid nitrogen for long periods of time to conserve genes and gene segments. DNA extracts, DNA or RNA sequences are considered genetic resources and are now routinely conserved in DNA banks (Adams 1997). The Royal Botanic Garden in Kew holds a DNA bank conserving more than 13000 samples of plant genomic DNA (http://www.rbgkew.org.uk/data/dnaBank/homepage.html).

Botanic Gardens, like field genebanks, maintain there plant material traditionally as living collections in the garden. While field genebanks are commonly used for crops and fruit trees, botanic gardens have a strong focus on wild species which are frequently endangered in their natural habitat (Heywood 1998; Wyse Jackson 1998). It is estimated that there are 2,204 botanic gardens known in the world, in 153 countries, conserving about 6.13 million accessions in their living collections.

***In situ* conservation**

For a more effective conservation of genetic diversity, it has now widely been recognized that *in situ* conservation should be regarded as a primary mode of conservation, complemented with *ex situ* collections. *In situ* methods involve the maintenance of genetic, species and ecosystem diversity, either in the wild or in traditional farming systems. It has been defined by Maxted & al. (1997) as the location, management and monitoring of genetic diversity in natural wild populations within defined areas designated for active, long-term conservation. Natural reserves or other forms of protected areas are most indicated to conserve wild species and wild relatives of crop plants. The target and mode of conservation is quite distinct for on farm conservation, where the scope is to maintain locally developed traditional crop varieties within traditional agricultural, horticultural or agri-silvicultural systems, with humans as the main driving force. The conditions in which the farmer can maintain genetic diversity of landrace varieties need therefore be promoted and preserved. For certains species such as fruits and vegetables, medicinal, and flavouring species, home gardens have also been recognized as a form of *in situ* conservation. They often serve as refuges for crop and crop varieties that were once more widespread in the larger agro-ecosystems and may also serve as sites for experimentation and introduction of new cultivars.

The above described *ex situ* and *in situ* methods all serve the common goal of plant genetic diversity conservation and are complementary sides of the same plant conservation coin. Depending on the target taxon, the aim of conservation and the resources available, one strategy or technique should function as a back-up to another. If the target taxon is for example a tuber crop such as potato, field genebanks and *in vitro* conservation will be predominant, backed up by seed and pollen conservation and wild relatives held in a genetic reserve. For a forest species producing orthodox seeds, both *in situ* conservation and seed storage would probably be equally important.

IPGRI, in collaboration with other organisations, has developed training guides, standards, handbooks and technical bulletins for most of the various *ex situ* and *in situ* conservation techniques. A few examples are genebank standards (FAO/IPGRI 1994), a protocol to determine seed storage behaviour (Hong & Ellis 1996), a training manual for the establishment of field genebanks (Mohd Said Saad & Ramanatha Rao 2001), Cryopreservation of *Musa* germplasm (Panis & Tienh Thinh 2001), and a training guide for *in situ* conservation on farm (Jarvis & al. 2000). A Guidelines for management of field and *in vitro* germplasm collections and technical bulletin on seed drying are currently in final stages of production. IPGRI, as many other international agricultural research centers, is therefore in a good position to collaborate and contribute its technical expertise to the conservation role of botanic gardens, becoming more and more involved in gene banking of seeds, micro-propagation and tissue culture, re-introduction programmes and natural reserve management (Hawkes & al. 2000)

Germplasm management and use

Over the past decenia of gaining experience with the development and operation of genebanks, the concept of germplasm management has become very important in the curation of germplasm collections. Whereas in the past the management of a collection was strongly focused on biological aspects of the material in question (i.e. understanding its flower biology; knowing how optimally germinate the seeds; defining the optimum seed moisture content for long-term storage; etc.), gradually the scope widened to include aspects of how to facilitate the use of conserved accessions (i.e. which activities to conduct to increase the utility of the accessions as well as understanding what the specific needs of the users of germplasm are), which germplasm material to include in the collection, what kind of conservation responsibilities (i.e. short- or long-term conservation responsibilities; *in situ* or *ex situ* approaches, or a combination thereof) to accept, whom to involve in the governance of the conservation efforts as well as how better to link conservation efforts to development.

Besides these largely biological questions, the application of technologies has become increasingly important, especially with the fast development of molecular genetic tools that can be used to screen germplasm collections, to search for genes, to assess genetic diversity within a given collection, etc. One key question that underpins the answers on most of these questions is related to the long-term cost of the conservation activities, including its routine operations, in order to be able to make sure that these costs can be met by (usually shrinking) budgets. Other more recent issues are related to questions on ownership over the germplasm material, intellectual property protection as well as the use of material transfer agreements and other legal aspects in exchanging germplasm. This evolution in the role of curators clearly demonstrates that a good genebank curator needs to be not only a good biologist/agriculturist but also a good manager, an economist, a lawyer, etc.

Considering the above, a brief account will be given of specific information and knowledge products that IPGRI has to offer to botanic gardens on the management and use of germplasm, especifically on important routine operations.

Organization of collections

A traditional way of organizing collections is based on the duration of the conservation combined with the utilization of the conserved germplasm, in particular with regard to the responsibility of distributing material or not. Long-term conservation is the principle basis for the so-called base collections whereas the utilization (through distribution) of the material is the overiding argument for the so-called active collections. Details of the operational and management aspects of these two types are presented by Sackville Hamilton & al. in Engels & Visser (2003).

Some germplasm collections have grown very big whereas for some crops numerous genebanks, dispersed around the world, maintain collections of the same genepool with a varying degree of duplication. To facilitate the use of such collections, Frankel (1984) suggested the establishment of core collections. A core collection can be defined as a collection of limited size with minimum similarity among its entries. It should represent maximum genetic diversity of a large collection, a crop, or a group of crop-related species. A core collection will always be substantially smaller than the collection(s) from which it derives. It may help in acquiring knowledge on the distribution of genetic diversity in the collection, the identification of gaps and priorities, and the potential use value of incorporated germplasm accessions in various environments, as well as apportioning responsibility among collection holders. Several IPGRI publications deal with the procedures to establish a core collection (van Hintum & al. 2000; Johnson & Hodgkin 1999; Hodgkin & al. 1995)

Monitoring of viability

Monitoring viability of stored seeds is important for conservation and much research has been carried out on germination and vigour testing. It is essential that the curator is able to assess accurately the initial viability of accessions prior to storage and then to monitor the viability of them during storage. Standards for viability monitoring in genebanks are included in the Genebank Standards (FAO/IPGRI 1994). Specific procedures for viability testing are included in the IPGRI handbooks on seed technology for genebanks, on principles and methodology (Ellis & al. 1985a), as well as on specific germination information and test recommendations (Ellis & al. 1985b).

Moreover, methods have been developed that require substantially less seed per test, e.g. sequential seed testing (Ellis & al. 1980).

Protocols have been developed at the International Seed Testing Association (ISTA) to test viability of numerous crop species (Ellis & al. 1985b). However, no specific management approaches for viability testing and monitoring have been developed and this has resulted in a range of practices being used. For specific species information on available protocols can also be obtained from botanical gardens and associations of seed producers. IPGRI is currently expanding the Seed Storage Behaviour Compendium developed jointly with Kew (http://www.ipgri.cgiar.org/themes/exsitu/seed_compendium.htm) with data on germination requirements and dormancy aspects. Determining seed vigour, in addition to germination percentage, could provide the genebank curator with early indications of a decrease in viability (Bewley & Black 1994).

Substantial differences may sometimes exist in longevity among accessions (i.e. germplasm samples) of the same species and even among genotypes within the same accession. It is therefore advisable to monitor seed viability carefully. Ensuring high viability of the stored samples reduces the need for regeneration, which is costly and has the risk of losing genetic diversity in the process. Therefore, it is recommended that each genebank develop its own monitoring procedures that guarantee effective and efficient conservation (Engels & Visser 2003).

Germplasm health

Infection and contamination of germplasm with pathogens may cause several problems in its management. The seed longevity may be affected, characterization and evaluation may be negatively influenced, pathogens may spread in the collection and destroy susceptible accessions, and pathogens may be distributed to new sites along with the germplasm. If not properly dealt with, infections will pose quarantine problems that negatively impact on germplasm flow. Adequate management measures should be taken by the genebank to eliminate infected germplasm accessions and to avoid new infections during the regeneration process. To facilitate genebank managers in this process, IPGRI is currently in the process of publishing a manual that addresses these issues (Morales in press) and recently published a germplasm health guide for the management of forest seed material (Sutherland & al. 2002).

Before being incorporated into an *ex situ* collection, samples need to be checked for presence of pests and diseases (Frison & Jackson 1995). In principle, all accessions intended for distributed must be healthy to limit the spread of diseases. This is particularly important in the case of vegetatively propagated crops, where there is a significant risk of spreading viruses, viroids and mycoplasmas. The genebank manager will have to devise an appropriate and detailed germplasm health monitoring strategy, taking into account national quarant ine regulations. This will often entail the use of diagnostic tools or the intensive inspection of plants grown from the seed stocks to be stored for conservation and utilization. Additionally, when involved in international transfer of materials, other protocols might be applicable. FAO and IPGRI have been working on establishing such protocols for several years and have issued a series of international guidelines for the safe movement of germplasm (SMOGS). The two most recent guidelines published include *Pinus* spp. (Diekmann & al. 2002) and *Acacia* spp. (Old & al. 2002). Specific techniques are available, in particular for the detection of virus infections, which allow detection and eradication of diseases from a number of species.

Characterization and evaluation

Characterisation and evaluation refers in the broadest sense to the description of material in the a collection (Hawkes & al. 2000). Characterizing accessions, an activity that is typically regarded as the responsibility of the genebank curator, involves determining the expression of highly heritable characters, ranging from morphological features to seed proteins and possibly including molecular markers. Such characters also enable easy and quick discrimination among phenotypes and allow simple grouping of the accessions, as well as a check on the trueness-to-type of homogeneous samples, frequently according to criteria used by breeders and other germplasm users. A well conducted characterization allows establishment of systematic relationships among accessions and even crops, including their evolutionary relationships. This would facilitate utilization of collections, allows detection of misidentifications and indicates possible errors made during other genebank operations (Bretting & Widrlechner 1995). It also results in better insight in the composition of the collecting and the coverage of genetic diversity. A proper characterization also makes an important contribution towards rationalizing management procedures, since it allows the curator to make well informed decisions on where best to regenerate the material (e.g. the japonica and indica rice races findings of Rao & Jackson 1996), to identify possible duplicates, to group germplasm accessions, etc.

Evaluation on the other hand covers the traits that are very useful for crop improvement, but which may often be affected in terms of their expression by the environment (Hawkes & al. 2000). The most valued traits in crop improvement include yield, agronomic performance, stress and pests resistances. It is obvious that an adequate evaluation of the collection represents an important prerequisite to effective use of the collection, as well as a major investment. The genebank manager should take every opportunity to get the conserved material evaluated. The expression of important traits is increasingly being researched using molecular markers (Bretting & Widrlechner 1995). Since this activity is not regarded by all genebanks as a typical genebank responsibility, but rather as a task for plant breeders and other users, close cooperation among all participants is essential to ensure useful application of results (Engels & Visser 2003).

Training and capacity building

The training and capacity development programme of IPGRI underpins the institute's first strategic choice to strengthen the capacity of countries, particularly of developing countries, to conserve, manage and use plant genetic resources sustainably for increased food security, environmental protection and improved livelihoods. Knowledge and technologies relevant to the improved conservation and use of PGR are developed and disseminated. Activities are designed and implemented together with partners, based on their own needs, in their own institutional system and within their own context. Capacity development also encompasses strengthening the larger system (e.g., policies and programmes) in which the organization operates, including the social, political and environmental context, and the financial and physical resources available to the organization. Capacity development also encompasses planning, coordination, decision-making and resource mobilization processes as necessary components of an effective organization.

Emphasis in the early years was on Master's level training, moving over time to more emphasis on individual skills-based training and short courses. Today, IPGRI's Training and Capacity Development Programme encompasses short-term training courses, provision of opportunities for MSc and PhD thesis research, support to the development of PGR courses or degree programmes in universities, study tours, individual on-the-job training, fellowship and internship schemes, and the development and dissemination of training materials.

Training and capacity building to help countries meet their own long-term training needs is in evidence throughout the programme and particularly conspicuous in curriculum and training materials development, training of trainers, and collaboration with developing country universities to develop regional Master's level courses. IPGRI's training capacity in terms of personnel is light but through drawing upon training inputs from partners, this capacity can be extended and given breadth and depth in subject coverage. A short course for example can draw upon training inputs from more than 10 research and training institutions, as well as funding from several sources. Developed-developing country trainer links are encouraged, particularly in relation to the development of regional university courses.

Over time, IPGRI's response to the need for training has evolved to reflect the changes based on experience and knowledge accumulated by national programme scientists and advances in the science of PGR. In recent years, there has been increased emphasis on the use of PGR; a broadening of the scope of conservation to include *in situ*, on-farm and integrated approaches to conservation; an expansion of the techniques for analysis of diversity, to include not only the gene level, but also the agro-ecosystem and forest ecosystem levels; more interest in the social and economic aspects of conservation and community participation; and a complication of the legal and policy framework relating to the conservation and use of PGR.

IPGRI Training and Capacity Development Programme is guided by a number of key principles. These are:

- Work with national programmes, particularly in developing countries;
- Know the target audiences and understand their needs;
- Empower partners to deliver; priority is given to activities that contribute to building sustainable institutional capacity at a national level (through local, regional and global approaches);
- Adopt participatory, learner-centered approaches;
- Seek strategic partnerships with national, regional and international organizations based on comparative advantage;
- Leverage and increase impact of training and capacity-building through ICT, networking, training of trainers and other approaches;
- Re-use, adapt, translate and upscale training materials to reach a wider audience.

Training courses

Up to now, over 6,000 scientists worldwide received training from IPGRI and its predecessor IBPGR in a wide range of topics on PGR conservation and use. In 2002 for example, 26 training courses have been organized or supported by IPGRI in which 525 trainees participated. IPGRI has conducted training on a wide ranging topics including for example: *ex situ* and *in situ* conservation techniques, characterization and seed multiplication, gender analysis and participatory approaches, poverty reduction in coconuts growing communities, Forest Tree seed collection and handling, conservation economics, use of molecular markers, policy frameworks, proposal writing and so on.

Training and reference materials

Reference manuals and training materials have been produced and distributed in various formats, media and languages. Management and decision-making tools for establishing and strengthening national PGR programmes have also been developed. Training materials are available in different languages on *ex situ* conservation, introduction and planning of collecting missions, on ecogeographic surveys, as well as on the measurement of genetic variation using molecular markers. Several training materials have been produced and are available for download:
http://www.ipgri.cgiar.org/training/download.asp

Technical guidelines and technical bulletins are also produced such as:

- A Training Guide for *In Situ* Conservation On-farm
- A protocol to determine seed storage behaviour
- Compendium of Information on Seed Storage Behaviour
- Establishment and management of field genebank
- *In vitro* collecting techniques for germplasm conservation
- Molecular tools in plant genetic resources conservation: a guide to the technologies

- Participatory approaches to the conservation and use of plant genetic resources
- Promoting the Conservation and Use of Plant Genetic Resources by Training and Research
- Technical guidelines for the safe movement of germplasm, now published for 20 crops.

The Guidebook for Genetic Resources Documentation: A self-teaching approach to the understanding, analysis and development of genetic resources documentation (Painting & al. 1995) is one of the most popular publication requested and downloaded from the web site.

Public awareness and education

The public awareness programme at IPGRI aims to build and sustain financial and institutional support for plant genetic resources activities worldwide by raising awareness among key target audiences of the role of these resources in sustainable development and food security. National institutions play the central role in promoting the conservation and use of plant genetic resources. IPGRI supports the development of national systems and provides information, advice on policy issues and training in key skills. Networks are also important mechanisms for encouraging the collecting, conservation, exchange, evaluation, enhancement and use of genetic resources. IPGRI promotes the sharing of ideas, methodologies, technologies and results through networks. It develops and supports regional and crop-specific networks. It also contributes to global networks on themes of importance across regions.

Beyond the immediate circle of IPGRI's partners lies the broader circle of people who benefit from the Institute's work. IPGRI's ultimate beneficiaries are the farmers, forest dwellers and other members of the rural community in developing countries. The people who support these primary producers and processors form a group of intermediate beneficiaries. This group includes plant breeders and other scientists in the public and private formal research sectors, and development workers in both the government and non-government sectors. Lastly, urban consumers benefit when IPGRI's work leads to the increased production and diversity of food and other commodities or to lower prices.

IPGRI’s role in public awareness is to ensure that research results and other relevant information is brought before the public with the intention of increasing understanding of, and support for, PGR research and IPGRI. Both strands include a considerable measure of capacity building in the following main areas:

- Undertaking capacity building programmes in IPGRI and national programme partners including communication skills training package
- Conducting awareness raising activities aimed at policy makers, donors, media, NGO’s and children about plant genetic resources issues
- Improving knowledge and information about genetic resources research and providing it to national programmes and other interested parties, including maintaining responsive news page under the IPGRI website

IPGRI produces series of public awareness publications such as:

- Annual Report
- *Geneflow,* an annual publication about the Earth’s plant genetic resources
- *Pgr,* a news sheet providing up-to-date information on IPGRI activities to donors and policy-makers
- Calendar (in six languages)
- Fact sheets that provides background information about PGR and IPGRI

Botanic gardens, visited by approximately 600 million visitors worldwide a year are uniquely placed to educate and raise awareness among their communities of the value of plant diversity and of the need to conserve it. This makes them an important model and partner from which IPGRI can learn as we search for new ways to link genebanks with local communities.

Information sources

Managing and communicating information is one of the eight areas of IPGRI's work. To promote the conservation and use of plant genetic resources, IPGRI helps national programmes to analyze and document their own collections and to gain access to information about the collections of others. For example, IPGRI has helped national genebanks in the Americas document and analyze their plant genetic resources information by training staff on documentation techniques, providing documentation tools and promoting the use of standards to facilitate data exchange and networking. It has also facilitated users' access to the germplasm conserved and facilitated germplasm exchange by making available information on over 200,000 accessions in a Directory of Germplasm Holdings in Latin America and the Caribbean. This data can be found also online in IPGRI's germplasm holdings database (http://www.ipgri.cgiar.org/germplasm/dbintro.htm), which holds information about *ex situ* collections world wide. It is one of IPGRI's databases, that contain meta information about plant genetic resources.

IPGRI is promoting the use of standards for plant genetic resources documentation and for the exchange of PGR data and information through crop descriptors and multicrop passport descriptors. It has published nearly 90 crop descriptor lists during the past 25 years. The crop descriptor lists provide an international format and a universally understood 'language' for plant genetic resources data. Each descriptor list represents an important tool for a standardized characterization system. The series is targeted at curators, breeders, scientists and users managing crop genetic resources collections. (See the list of descriptors at http://www.ipgri.cgiar.org/publications/pubseries.asp?id_serie=13). The List of Multi-crop Passport Descriptors (MCPD) is a reference tool developed jointly by IPGRI and FAO to provide international standards to facilitate germplasm passport information exchange across crops. These descriptors aim to be compatible with IPGRI crop descriptor lists and with the descriptors used for the FAO World Information and Early Warning System (WIEWS) on plant genetic resources.

Exchange of information is as well one of the areas of work included in the Memorandum of Understanding (MOU) that has been signed in April 2001 between BGCI and IPGRI to formalize their partnership. The aim of this partnership is to advance the conservation and sustainable use of biodiversity by encouraging cooperation between botanic gardens and agricultural research centers. In order to facilitate and promote the exchange of information with botanic gardens IPGRI has developed some web pages, that will soon be published on IPGRI web site at http://www.ipgri.cgiar.org/themes/exsitu/Botanicgardens/botanicgardens_home.htm. The intent of this web page is to provide publications and other information produced by IPGRI in close collaboration with its partners that are both of relevance and intended to complement the genetic resources conservation work of botanic gardens. The pages are subdivided into PGR conservation relevant areas, and for each area you are provided with a short introduction and a list of IPGRI publication, training materials and other information resources.

Conclusion

In conclusion, there can be much to learn from the experience and progress made in the area of conservation and use of plant genetic resources by agricultural centres that could benefit the Botanic Garden community. Although the focus of agricultural centres in the past has been on crop plants and their immediate close relatives, there is an increasing recognition of the importance of conserving more distant relatives and even species and genera, as the barriers in conventional breeding practices are overcome by new technologies. Conserving wild crop relatives is becoming as important as conserving old landraces and varieties of crop. There is need for more research to be conducted on wild plants species to better understand their biology and to develop technologies and strategies for their conservation. This has become more urgent as wild species are becoming more and more threatened and it will require greater collaborative efforts to halt the rate at which species and genetic diversity are being loss. There is need for the networks among Botanic Gardens and Agriculture centres to join forces and exchange information and we hope that the IPGRI web page dedicated to botanic gardens will be one of the means in achieving this aim.

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Seed banking at the National Botanic Garden of Belgium

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Abstract.. *– Seed banking at the National Botanic Garden of Belgium concerns the* ex situ *conservation of seeds coming from (1) the living collections of the garden, (2) wild-collected native species of Belgium and (3) the collection of wild representatives Phaseoleae–Phaseolinae. Information is given on source, storage, use, databank and documentation.*

Key words*: seed conservation,* ex situ *conservation*

1 Introduction

As many other botanic gardens and arboreta in the world, the National Botanic Garden of Belgium (N.B.G.B.) is envolved in *ex situ* conservation. This one refers to the maintenance of samples of plants or seeds.

Seed banking has undoubtedly advantages over the conservation of entire plants, particularly for annuals and short-lived perennials. It allows the storage for a very long period of a high number of large seed samples requiring little space and relatively few labour. The major difficulty encountered in the management of a seed bank is to dispose of appropriate seed monitoring methodologies because of the very large diversity regarding storage, germination, dormancy, ...

Seed banking at the N.B.G.B. only concerns "orthodox" seeds. They can be dried to low moisture contents and stored at low temperatures without loss of viability and genetic integrity. "Recalcitrant" seeds, which are killed by drying or freezing, cannot be stored under the standard conditions.

The seed bank is equipped with a cold room (50 m^2) where the temperature and relative humidity are maintained respectively at 15 °C and 10 %. These conditions are quite suitable for short-term storage (10 years), the seeds being conserved in open glass or paper containers placed on shelves. For long-term storage, seeds dried at 5 % moisture content are packed in sealed plastic-aluminium laminated bags and placed in deep-freeze units at -20 °C.

Seeds manipulated in the seed bank of the N.B.G.B. can be classified in three groups according to their provenance: seeds collected in the living collections of the garden, seeds of native species collected in the wild in Belgium, and seeds of our germplasm collection of wild representatives of Phaseoleae –Phaseolinae.

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2 Living Plant Collections

Seeds are collected from plants cultivated in the collections, mainly outdoors. About 2000 accessions are conserved on a short-term basis.

The seed material is useful for the sowing programmes of the N.B.G.B. About 600 seed samples, mainly of annuals and biennials, are required every year. The surplus of seeds is offered for exchange with other gardens or scientific institutions. More than 3,500 samples are annually dispatched to 300 institutions.

The database, named LIVCOL, is a home-made databank using PROGRESS software for the management of the whole living plant collections. It includes modules for seed monitoring (harvest date, action, priority to harvest, ...), seed dispatching (mailing, accessions, documents, dispatching date, ...), the own seed catalogue (years, accessions, requested items, dispatched items, ...), seed catalogues from other institutions (years, donor's conditions, medium, ...), seed bibliography (authors, year, title, review, keywords, ...).

The seeds available for distribution are presented in an annual seed catalogue (Anonymous 2002a). It includes about 750 accessions, from which 70 % come from the outdoor collections, 30 % are of known origin and 75 % are from recently verified plants. The seed catalogue is dispatched to 550 regular correspondents. It can also be consulted on the web site of the N.B.G.B. (Anonymous 2002a).

3 Indigeneous flora of Belgium

Seed samples from wild plants indigeneous in Belgium were collected between 1989 and 1997 (Billiet & al. 1990, Vanderborght 1993). Seeds of some 650 accessions, from 450 taxa, are stored on a long-term basis. 176 accessions (30 % of the collected material) have been duplicated in 2002 at the Millenium Seed Bank (Wakehurst, U.K.) in order to achieve a higher level of guarantee of safeguard of the biodiversity conservation and to carry out germination tests.

The *ex situ* conservation of seeds of wild plants is viewed as a tool which can be useful to an integrated conservation strategy. The main objective of the seed collecting programme is the long-term conservation of representative or endangered populations coming from different phytogeographical parts of Belgium. The stored packets of seeds can be used as sources of material for population reinforcement or reintroduction programmes. They are also put at the scientific community's disposal for research activities in the fields of germination, conservation, dormancy, ... Seed material of a limited number of accessions has been used to be planted in the outdoor collections of the N.B.G.B.

The above-mentioned database LIVCOL includes a module for the seed monitoring (harvest date, duplication, ...), seed bibliography (authors, year, title, review, keywords, ...).

4 Collection of wild Phaseoleae–Phaseolinae

The N.B.G.B. since 1988 assumes the management of a wild Phaseoleae-Phaseolinae germplasm collection (Vanderborght & Baudoin 1998). The collection was established in 1965 by G. Le Marchand and R. Maréchal at the Faculty of Agricultural Sciences at Gembloux (Belgium). It has been designated by the "International Board for Plant Genetic Resources" (I.B.P.G.R.), now the "International Plant Genetic Resources Institute" (I.P.G.R.I.), as a base collection for wild species of *Phaseolus* (in 1979) and *Vigna* (in 1983). Original accessions are entered into the collection through exchange or gift. Seeds for renewal are collected on plants cultivated in glasshouses.

The collection covers a very wide genetic diversity. It currently contains 1617 accessions representing 202 taxa of the Phaseoleae tribe, chiefly centered on the Phaseolinae subtribe. *Phaseolus* and *Vigna* are the best represented genera with respectively 30 species (688 accessions) and 61 species (755 accessions). The largest number of accessions are wild or weedy materials, originating from 87 countries.

The main objective of the collection is to secure long-term conservation in the form of seed samples. Some 3,000,000 seeds are stored. In order to increase the security, the genetic material is duplicated in two different places at the Garden.

The collection provides the basic material for the investigations conducted in scientific institutions in the fields of taxonomy, genome analysis, definition of reservoirs, agronomic and chemical evaluations, interspecific and plant breeding, ... About 400 seed samples are dispatched per year.

A home-made databank using PROGRESS software, called PHASEO, is used for data manipulation related on source, origin, cultivation, morphological characterization, identification, phenology, documentation. Seed monitoring data are also included, such as harvest and banking date, seed quantities, germination results, ...

The list of taxa for which genetic material can be obtained is published in the annual seed catalogue (Anonymous 2002a) as well on the web site of the Garden (Anonymous 2002b).

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First year report of Trentino Seed Bank project (NE Italy)

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Abstract. – *Trentino Seed Bank Project started in summer 2002 and intends to contribute to* ex-situ *plant conservation in the SE Alps, carrying out an extensive seed collecting campaign and developing a regional, long-term seed storage facility. The good knowledge of plant distribution in Trentino allowed to select with confidence 73 top priority species and to target the actual populations to be sampled. In a three year period two teams aim to collect in the field approx. 400 seed accessions, supplemented by an assessment of the population size and fertility. In summer and autumn 2002 each team visited more than 80 sites and collected more than 50 seed samples, recording accurately all geographic data on the sites visited. Full details on the collections made in 2002 are given. The Seed Bank itself is essentially composed of a geographic resource office equipped with all technical local maps, GPS and GIS facilities; a desiccation chamber equipped with sorption driers in conjunction with a cooling system, an air-lock and a monitoring system, a seed cleaning and testing lab, incubators and two deep freezers that serve as a cold store.*

Key words: Ex situ *conservation, seed bank, endangered species*

Abbreviations*: CFT, Cartografia Floristica Tridentina (Trentino Plant Distribution Mapping Project); RH, relative humidity*

1 Introduction

The Alps as a whole are the principal centre of plant diversity and endemism in Europe (Heywood & Davis 1994), hosting approx. 5,500 species of vascular plants (43% of the European flora) (Ozenda 1985, Aeschimann & al. 1991), 350 of which are endemic (Pawlowski 1970) being the 7% of the total Alpine Flora. Within the whole chain three major centres of endemism can be noted and the largest one is located in the South eastern Alps (Heywood & Davis 1994), the area where Trentino lies. This zone is therefore worth a special conservation effort. Among the 2,359 species occurring in our region 723 (the 30,6%) are at risk according to the newly published regional red data book (Prosser 2001). To contribute to plant conservation in our region, the Alpine Botanical Garden 'Viotte' in 2001 set up the Trentino Seed Bank Project; subsequently the Trentino Seed Bank was established in summer 2002 (Bonomi & al. in print). This project aims to carry out an extensive seed collecting campaign and to

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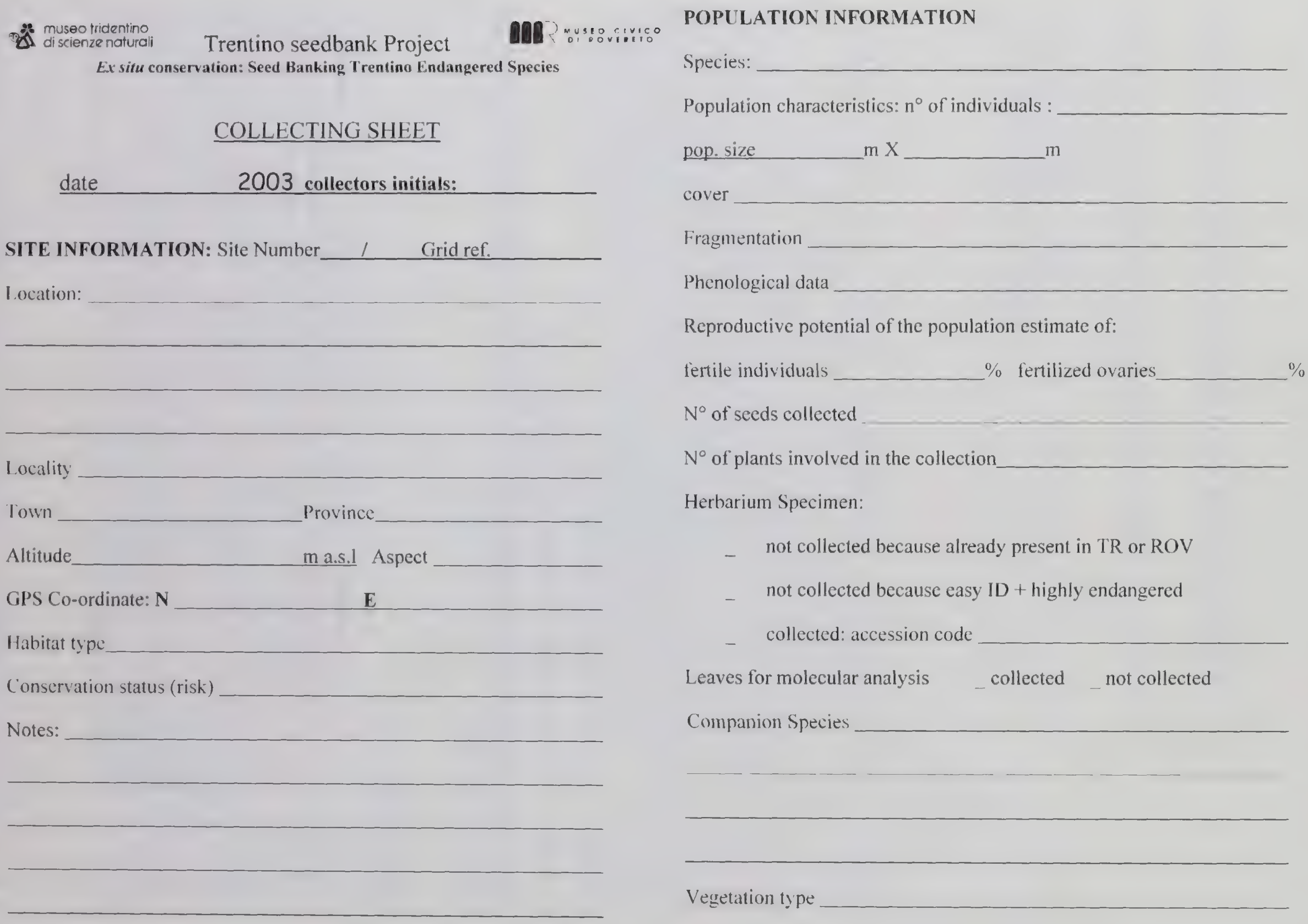

museo tridentino di scienze naturali

Trentino seedbank Project

MUSEO CIVICO DI ROVERETO

Ex situ **conservation: Seed Banking Trentino Endangered Species**

COLLECTING SHEET

date ____________ 2003 **collectors initials:** ____________

SITE INFORMATION: Site Number ____ / ____ Grid ref. ________

Location: ____________________________

____

____

____

Locality ____________________________

Town ______________ Province ____________

Altitude ______________ m a.s.l Aspect ____________

GPS Co-ordinate: **N** ______________ **E** ______________

Habitat type ____________________________

Conservation status (risk) ____________________

Notes: ____________________________

____

____

____

____

POPULATION INFORMATION

Species: ____________________________

Population characteristics: n° of individuals : ____________

pop. size ________ m X ________ m

cover ____________________________

Fragmentation ____________________________

Phenological data ____________________________

Reproductive potential of the population estimate of:

fertile individuals ________ % fertilized ovaries ________ %

N° of seeds collected ____________________________

N° of plants involved in the collection ____________________

Herbarium Specimen:

- _ not collected because already present in TR or ROV
- _ not collected because easy ID + highly endangered
- _ collected: accession code ____________

Leaves for molecular analysis _ collected _ not collected

Companion Species ____________________________

____

____

____

Vegetation type ____________________________ develop a regional, long-term seed storage facility. The project is complemented by minor tasks experimenting propagation and re-introduction of five target taxa, and assessing the genetic diversity of a sub sample of the target species. These minor tasks will however not be described in this paper.

2 Material and methods

The nomenclature of the taxa cited in this paper complies with the current edition of the International Code of Botanical Nomenclature (Greuter & al. 2000) and the taxonomic treatment follows Ehrendorfer (1973), with the exception of *Primula* sect. *Auricula*, here according to Prosser & Scortegagna (1998), and the *halleri* group of the genus *Festuca* that follows Pils & Prosser (1995).

2.1 Monitoring plant populations

Although the distribution of plants in Trentino can be considered sufficiently known, new discoveries cannot be excluded (Prosser & Scortegagna 1998). Monitoring the distribution of the endangered species provides important information on population size over time and points out new species that may need active protection. To attain this, poorly investigated areas are being further explored and old records are being checked. The plant mapping activity complies with the standard of the Central European plant distribution mapping project (Ehrendorfer & Hamann 1965) employing the MTB grid (Meßtischblatt quadrant of 3' lat x 5' long). The data recorded during the field work is stored in the already structured regional plant distribution CFT database (Festi 1994) and is linked to a GIS System to produce synthetic and predictive models employing MapInfo and MapX softwares. If species of difficult identification are found, an herbarium voucher specimen is collected to be sent to specialists. The constant updating of plant distribution records also plays a key role to assess the eventual decline of a given species over time and to point out new species that may need active protection.

2.2 Establishing priority for seed collection

In Trentino the good knowledge of plant distribution (thanks to ten years of surveys carried out by the CFT, the regional plant distribution project; Prosser & Festi 1992, 1993; Prosser 1993, 1994, 1995, 1996, 1999, 2000, 2001) allows the pinpointing of the species that are running an high risk of extinction and need conservation. It is therefore possible to prepare a detailed list of the endangered species collating data from the world (Walter & Gillet 1998), the Italian (Conti & al. 1992) and the local red data book (Conti & al. 1997; Prosser 2001) based on actual cartographic records and data. All species endangered at world level and occurring locally were considered supplemented by a selection of other species endangered locally and elsewhere rare in Europe and Italy together with all narrow endemic species. For each species up to ten sites (if existing) were selected in order to maximise the genetic diversity of the samples to be collected.

2.3 Seed Collecting

Even if to encompass rare alleles in a population few hundreds seeds could be enough (Lawrence & al. 1995), seed banks require at least a few thousands seeds in order to test and propagate them (Ellis & al. 1985). However during collection a population is never deprived of more than 20 % of its seeds so that its reproductive potential is not significantly affected (Marshall & Brown 1975; Guarino & al. 1995). The collecting bags employed are made of breathable fabric (Smith 1995). When a site is visited, full data on its geographical position is carefully recorded. An estimate is given of the extension of the population (rough figure in m^2), its cover value, sociability and reproductive potential (% of flowering individuals and % of fertilized ovaries). The collecting sheet employed is shown in fig. 1.

2.4 Seed treatment and storage

In order to guarantee their long term conservation, the seeds' moisture content is lowered to values ranking between 3% and 7% (Black & Pritchard 2002). The seeds are slowly dried for several months at 20% RH and 15°C in a dry room actively conditioned (Smith & al. 2003). The seeds are then cleaned recording information on their number and moisture status. They are then packaged in moisture proof containers and stored at –20°C in deep freezers.

→

Figure 2. Callianthemum kernerianum *and its seeds, a narrow endemic species occurring only on Monte Baldo (IUCN status VU). Site: Cima del Longino, 15th May 2003.*

Figure 3. Orchis spitzelii, *a very rare species occurring only in three sites in Trentino (IUCN status CR). Site - Val Brenta, Dolomiti di Brenta, 12th June 2003.*

Figure 4. Dracocephalum austriacum *and its seeds, a very rare species occurring only in two sites in Trentino (IUCN status EN). Site: Monte Malachino, 28th May 2003, 27th July 2002.*

←

Figure 1.
The collecting sheet employed in the seed collecting activity.

3 Results

3.1 Monitoring plant populations

A total of 22 field trips were carried out in summer 2002 as detailed in appendix 1. The monitoring team worked for a total of 277 contact hours equivalent to 37 working days. Twenty five grid squares were investigated for a total of 7,893 total species recorded, 801 of which represent new records for the visited grid squares. A total of 3,251 km were travelled by car and an ascent of 15,663 m was covered on foot.

Unfortunately no old records of rare plants could be confirmed. In Talpian (Val Duron, Campitello) the 22th of July the rare *Carex heleonastes* and *C. capitata* could not be found; in Val Malgazza (Bresimo) the 30th of July *Botrychium simplex, B. matricariaefolium,* and *B. lanceolatum* were not ascertained and the 31st in Limes (Val Daone) *Botrychium virginianum* could not be found either. The 21st of August the search for *Peplis portula* at Laghestel di Pinè was fruitless. These unconfirmed records suggest that the surveys carried out in the last eleven years of the regional plant distribution mapping project attained a good cartographic detail. The likely disappearance of these rare species recorded of old in our area is however an important information. It indicates that some environmental changes happened in our region and negatively affected the presence of rare plants in Trentino.

However during the present investigations some unexpected and notable species could also be recorded. The most significant ones are the discovery of *Minuartia laricifolia* in the Lagorai group on Mt. Fornace, being the first record for the eastern part of our region. Moreover in two sites (Lago di Lases and Lago della Serraia) the hybrid *Equisetum* x *litorale* (*E. arvense* x *fluviatile*) was recorded. This hybrid species was never recorded before in Trentino.

3.2 Establishing priority for seed collection

The selection of the target species according to the criteria exposed in 2.2 produced a list of 73 top priority species being 3.1% of the local flora, including all the narrow endemic species and all the species endangered at world level, being 7.6% of the locally endangered species and 1.1% of the other non endangered species. The detailed list can be found in Appendix 2. In this list the species appear in blocks of decreasing priority: firstly twenty two maximum priority species, endangered in the world and occurring in Trentino such as *Callianthemum kernerianum* (fig. 2); followed by four taxonomically critical species reported as endangered in the world and occurring in Trentino; one species reported as endangered in the world and occurring in Trentino, but considered not in danger according to the most recent data; nine species critically endangered in Trentino, (IUCN status CR) that occur in less than three Italian regions, such as *Orchis spitzelii* (fig. 3); twelve species endangered in Trentino (IUCN status EN) that occur in less than three Italian regions, such as *Dracocephalum austriacum* (fig. 4); seven other rare species endangered in Trentino (various IUCN categories) particularly worthy of conservation; one species listed in appendix II of EU Habitat directive, endangered and occurring in Trentino; seventeen Trentino narrow endemic species. With respect to the original list supplied in the Seed Bank application dating back to September 2001 (Bonomi unpubl.), this present list has been extended to include seven additional species that were found to be of further particular regional and national interest according to the most recent investigations carried out in the last two years. The list now includes 73 species for a total of 434 sites selected.

3.3 Seed collecting

The first year collecting campaign started in July and ended in November 2002. The four people in the collecting teams worked for a total of 808 hours in 83 collecting trips, travelling 11,519 km by car and ascending on foot a minimum of 31,349 m in order to visit 186 sites (thirty of which were visited more than once) and collect one hundred seed accessions. Further synoptic details can be found in fig. 5 and in tab. 1 and 2.

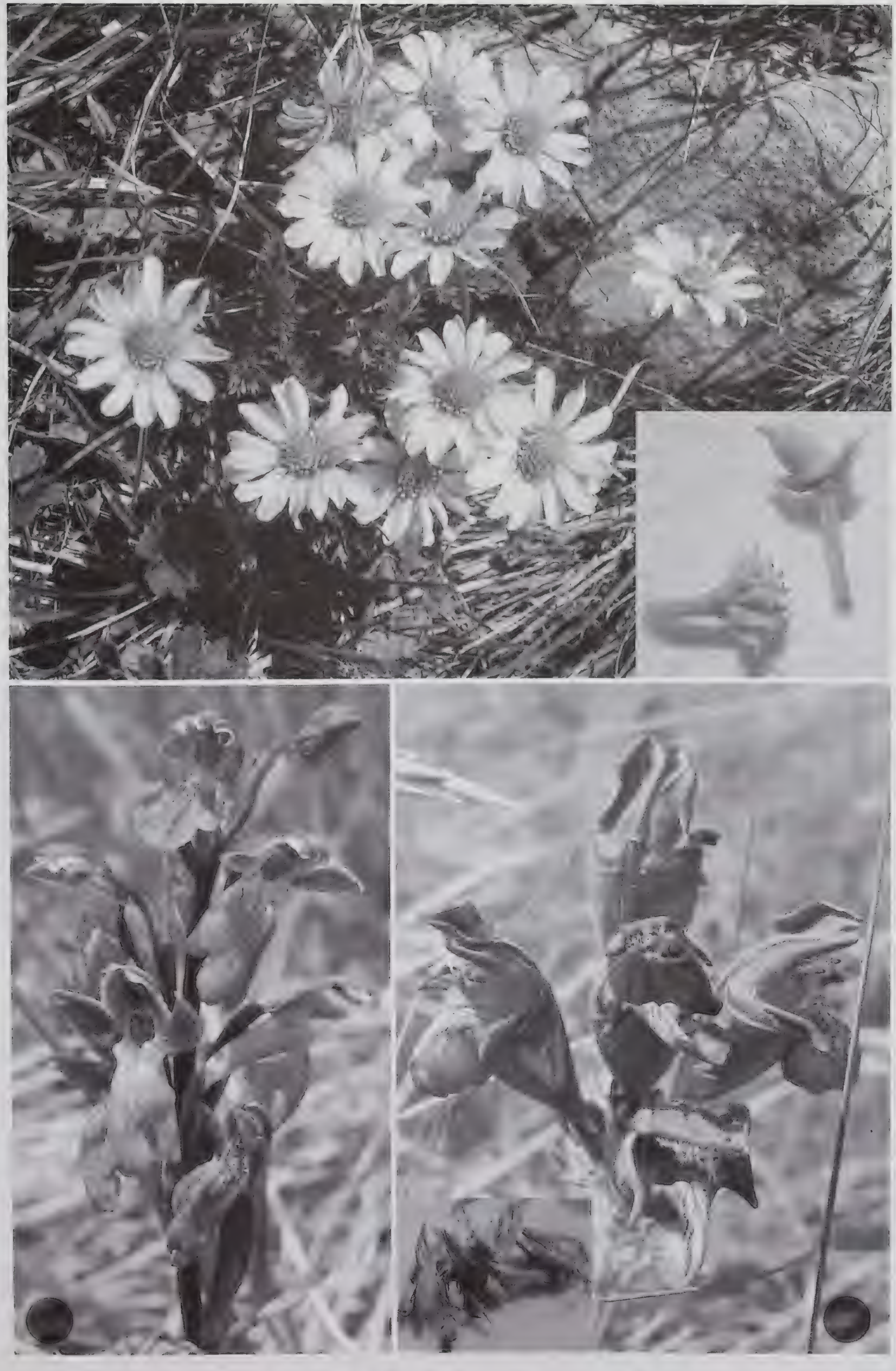

Figure 2-4. Callianthemum kernerianum, Orchis spitzelii & Dracocephalum austriacum *(caption page 103)*

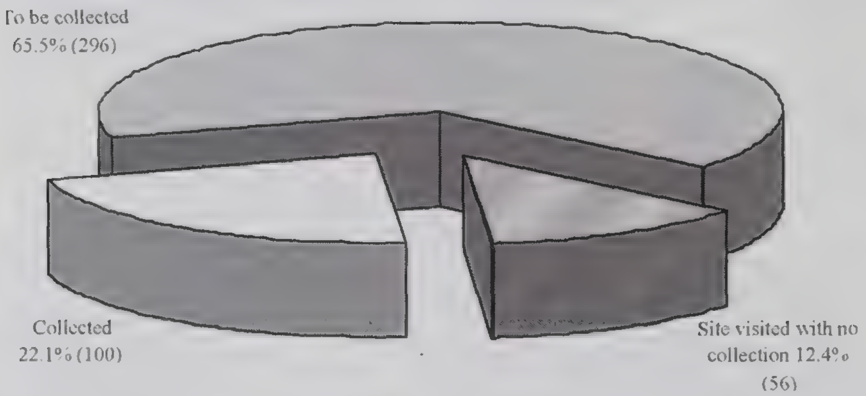

Figure 5. Pie chart showing the proportion of sites visited and collections carried out in the first year collecting campaign (2002).

Table 1. Details of the seed collecting effort

Total hours	**808**
Day trips	**83**
Mean duration of day trip	**9 h**
Total ascent on foot	**31,349 m**
Total km travelled by car	**11,519 km**

Table 2. Details of the seed collecting results (in brackets number of sites not visited more then once)

Sites visited	**186 (156)**
Sites where seeds were collected	**111 (100)**
Sites where seeds could not be collected because plants not yet mature or sterile	**37 (35)**
Sites where target species could not be found	**38 (37)**

During the collecting campaign the phenological stages of the local populations were also recorded. These data very often differ significantly from the information that could be gained from the literature. It must however be noted that the information usually available concerns the flowering time and not the time when the fruits are mature and the seeds ready for collection. This time can only be inferred considering a standard ripening time. This is however much variable from species to species. According to our observations, in the genus *Daphne* the seeds are usually ready to be collected 15-20 days after the flowering maximum, on the contrary in the genus *Primula* and *Cypripedium* over three months must pass after the flowering time for the seeds to be ready for collection.

Many collecting trips (roughly 30% of the total) were not successful because the site was visited when the seeds were not yet ready for collection or when they had already been dispersed. These trips were nevertheless useful to determine a more reliable fruiting time. Globally the collected season turned out to be much longer than expected. The seeds of the different target species selected could be collected from May to November with a peak collecting period in August and September.

All the information gathered during the collecting campaign will be progressively accessioned in a database allowing in this way to evaluate the effect of altitude and geographic location on the seed ripening time between different populations of the same species and also different species.

3.4 Seed treatment and storage

In the first year of activity the seed bank facilities have been designed and put into operation. The Trentino Seed Bank is essentially composed of a geographic resource office, a dry room and cold store and a seed curation lab. The geographic resource office includes all technical equipment necessary for the field trips, (incl. local maps, field aids such as hand lenses, excursion floras, collecting bags, altimeters, GPS, digital cameras, laptop and a PC workstation equipped with a GIS). The dry room is designed to host newly collected seeds in stackable baskets and lower their moisture content in a non destructive way. The seeds are kept at low humidity employing a sorption drier in conjunction with a cooling system maintaining a controlled environment at values of 15% RH and 15°C. It is well insulated by the thick walls (more than one meter thick) of the Medieval building where it is hosted; its inner surface is tiled for further insulation. It is equipped with an air lock to allow staff in and out with a minimal disturbance to the dry conditions. Temperature and humidity loggers constantly monitor the whole process and an alarm system warns of any malfunctioning. The seed treatment lab includes a seed cleaning section with a seed blower and sieves under a dust extracting cabinet, a high magnification dissecting microscope, a precision four digit analytical balance and a fan assisted oven, to collect preliminary curation data. A second part of the lab has been designed to serve seed packaging and is equipped with a Hydrolytic RH sensor with the probe contained in a airtight container to measure RH generated by the seed at equilibrium (i.e. the water activity) and get an indirect non destructive measurement of the seed's moisture content. Packaging containers available include foil bags with a heat sealer, clamped glass jars and glass bottles. As a safety precaution, double packaging is used and the seals are monitored with silica gel indicators, previously equilibrated with dry room conditions, included within the containers. The seed viability section is equipped with chemicals for cut tests and two cooled incubators for germination/viability tests. The long term cold storage is currently entrusted to two conventional deep freezers, that represent a modular option, being very easily substituted and increased in number should any malfunctioning occur or more room be required. Seed germination testing will be progressively implemented in future years.

4 Discussion

According to schedule, during the first year of the project, 1/3 of the sites (33.5%) was visited and, in 64% of them, seeds were successfully collected. 20% of the sites needed to be visited more than one time, mainly because the seeds were not yet ready for collection at the time. In a minor number of cases the plant could not be found at all.

The information on the correct time of seed maturity gained during the first collecting campaign will be invaluable to attain an increased efficiency of collection in the future years. From a preliminary assessment of the data collected on the population size and fertility it can be noted that isolated population of few individuals tend to have very low fertility and many sterile or aborted seeds. For example let us consider the case of *Dracocephalum austriacum*. This is a Bern convention species, only found in Trentino in two small populations of a few thousands individuals each, few kilometres apart. Approx 5% of the seeds borne by these two populations were fully developed and viable and the remaining seeds were empty or not properly developed.

Another interesting species is *Callianthemum kernerianum*. This is a narrow endemic species, only found in Trentino on Mt. Baldo with three distinct populations of a few thousands individuals each, few kilometres apart. Approx 10% of the seeds borne by these populations were fully developed and viable and the remaining seeds were not properly developed.

When targeting different sites for collection we had no information regarding the population size and fertility, and the sites were chosen from different geographical locations and different altitude. However during the collecting campaign, some sites were found to be made up by a very limited number of individuals and some times also sterile. These sites were of course not suitable for seed collection. In some cases there was no alternative, the target species only grew in few and scarce populations, and no other site was available. However in some instances this happened also for species where more than 30 sites in the region were known. These sites with scarce and low fertility populations often occurred to the limit, either altitudinal or geographical, of the plant distribution area. In the attempt to maximise the genetic diversity of the planned collection some selected populations were unsuitable for collecting purposes.

On the basis of this information in the next autumn the selected sites will be critically reviewed trying to re-select other sites that might not present this inconvenient.

Occasionally when collecting a given species other target species were found and their seeds collected, if ready. These supplementary collections were nevertheless useful and might stand in place of sites unsuitable for collection.

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Appendix 1. Details of the plant distribution monitoring activity

date	Grid Ref.	Location	Rilevatori	Total species recorded	New species in the grid	ascent on foot	Km trevelled	working hours
11/6/2002	0031/3	Varignano (Arco)	Prosser, Sarzo, Maffei	253	9	240	48	5.15
22/7/2002	9536/1	Tal Pian (Val Duron)	Prosser	139	3	62	248	13.30
26/7/2002	0033/4	Valle Lunga (Val d'Astico)	Prosser	363	51	765	66	10.50
30/7/2002	9531/3	Val Malgazza	Prosser	212	12	898	169	12.00
30/7/2002	9531/4	Val Malgazza	Prosser	259	25	378		
31/7/2002	0029/1	Redoten (Val Daone)	Prosser	317	27	850	165	14.00
2/8/2002	0028/2	Grapperi di Nuova (Val Daone)	Prosser, Zara	113	12	170	175	14.25
19/8/2002	9835/4	Quarazza (Lagorai)	Prosser	236	16	876	155	13.30
20/8/2002	0231/2	Bocca d'Ardole (M. Baldo)	Prosser	388	14	1095	35	12.30
20/8/2002	0231/4	Bocca d'Ardole (M. Baldo)	Prosser	207	6	130	\	\
21/8/2002	9833/3	Altipiano di Pinè, etc.	Prosser	462	64	251	102	13.30
26/8/2002	9833/3	Lago di Lases (Val di Cembra)	Prosser	424	30	287	95	12.00
27/8/2002	0231/2	Colme di Pravecchio (M. Baldo)	Prosser	456	28	625	55	13.00
28/8/2002	9834/2	Val Trigona (Lagorai)	Prosser	116	4	65	150	13.00
28/8/2002	9834/4	Val Trigona (Lagorai)	Prosser	244	19	610	\	\
30/8/2002	9834/2	Val Cadino-Cima Fornace (Lagorai)	Prosser, Zara	321	31	845	199	13.20
2/9/2002	9835/2	Caoria-Cima Spiadon (Lagorai)	Prosser, Zara	250	67	1346	261	14.15
3/9/2002	9833/4	Lago della Serraia, Dosso di Costalta	Prosser	402	48	936	99	12.40
4/9/2002	0031/2	Laghisoli, Val Deserta (Dro)	Prosser	551	86	1260	84	12.40
5/9/2002	9931/4	M. Casale	Prosser, Zara	433	50	440	121	12.30
6/9/2002	9732/2	Priò, forra del Pongaiola, Val Marzana	Prosser	438	28	785	128	12.55
10/9/2002	9834/1	Rio Valletta-M. Croce (Lagorai)	Prosser	287	39	1029	196	13.35
11/9/2002	9934/1	Hoabonti-7 Laghi (Lagorai)	Prosser	335	59	830	126	12.40
17/9/2002	9836/4	Schener, Paneveggio (Primiero)	Prosser, Perazza	437	58	340	334	12.30
12/11/2002	9836/2	Val Lozen, Osne (Primiero)	Prosser, Zara	340	15	550	240	12.30
		Total		7,983	801	15,663	3,251	277.05

Appendix 2. Trentino Seed Bank Target Species in blocks of decreasing priority.

Species listed in appendix II of EU Habitat directive: preceeded by ***
Narrow endemic species occurring in Trentino: underlined
list format: *Genus species* author **/sp code/N. of sites selected - available**

Maximum priority: species endangered in the world and occurring in Trentino (22)

1. *Aquilegia thalictrifolia* Schott & Kotschy/**AT/10 sites**
2. *Artemisia nitida* Bertol./**AN/10 sites**
3. *Callianthemum kernerianum* Freyn ex A. Kerner /**CK/10 sites**
4. *Campanula morettiana* Rchb./**CM/10 sites**
5. *Campanula raineri* Perp./**CR/4 sites**

*** 6. *Daphne petraea* F. E. Leyb./**DP/10 sites**

7. *Daphne reichsteinii* Landolt & E. J. P. Hauser/**DR/7 sites**
8. *Fritillaria tubaeformis* Gren. & Godr./**FT/3 sites**
9. *Gladiolus palustris* Gaudin/**GP/10 sites**
10. *Nigritella buschmanniae* Teppner & Ster/**GP/6 sites**
11. *Primula daonensis* (Leyb.) Leyb./**PD/10 sites**
12. *Primula glaucescens* Moretti/**PG/4 sites**
13. *Primula recubariensis* Prosser & Scortegagna/**PR/5 sites**
14. *Ranunculus bilobus* Bertol./**RB/10 sites**
15. *Rhizobotrya alpina* Tausch/**RA/10 sites**
16. *Saxifraga arachnoidea* Sternb./**SA/8 sites**
17. *Saxifraga facchinii* Koch/**SF/10 sites**

*** 18. *Saxifraga tombeanensis* Boiss. ex Engler/**ST/10 sites**

19. *Saxifraga vandellii* Sternb./**SV/10 sites**
20. *Silene elisabethae* Jan/**SE/9 sites**
21. *Telekia speciosissima* (L.) Less./**TS/2 sites**
22. *Viola dubyana* Burnat ex Gremli/**VD/10 sites**

Taxonomically critical species reported as endangered in the world and occurring in Trentino (4)

23. *Knautia baldensis* A. Kerner ex Borbás/**KB/9 sites**
24. *Knautia persicina* A. Kerner/**KP/10 sites**
25. *Knautia velutina* Briq. I/**KV/10 sites**
26. *Sempervivum dolomiticum* Facchini R/**SO/8 sites**

Species reported as endangered in the world and occurring in Trentino, but considered not in danger according to the most recent data (1)

27. *Physoplexis comosa* Schur R/**PC/10 sites**

Species critically endangered in Trentino (IUCN status CR that occur in less than 3 Italian regions (9)

28. *Allium lineare* L./**AR/1 site**

*** 29. *Botrychium simplex* E. Hitchc./**BS/1 site**

30. *Botrychium virginianum* (L.) Sw./**BV/1 site**
31. *Carex randalpina* B. Walln./**CL/1 site**
32. *Hieracium alpicola* Schleicher ex Steudel et Hochst./**HA/2 sites**
33. *Juncus arcticus* Willd./**JA/5 sites**
34. *Orchis spitzelii* W. D. J. Koch/**OS/3 sites**
35. *Typha shuttleworthii* W. D. J. Koch & Sond./**TH/1 site**
36. *Viola elatior* Fr./**VE/1 site**

Species endangered in Trentino (IUCN status EN) that occur in less than 3 Italian regions (12)

37. *Androsace lactea* L./**AL/4 sites**
38. *Carex appropinquata* Schumach./**CA/4 sites**
39. *Carex disticha* Huds./**CD/5 sites**
40. *Carex norvegica* Retz./**CN/1 site**
41. *Carex pulicaris* L./**CP/7 sites**
42. *Crepis mollis* (Jacq.) Asch./**CO/1 site**
43. *Dianthus glacialis* Haenke/DG/**2 sites**

*** 44. *Dracocephalum austriacum* L./**DA/2 sites**

45. *Ephedra helvetica* C. A. Meyer/**EH/1 site**
46. *Hieracium caespitosum* Dumort./**HC/1 site**

*** 47. *Liparis loeselii* (L.) Rich./**LL/6 sites**

48. *Orobanche elatior* Sutton/**OE/3 sites**

Other rare species endangered in Trentino (various IUCN categories) particularly worthy of conservation (7)

49. *Androsace wulfeniuana* W.D.J. Koch /**AW/2 sites**
50. *Lathyrus palustris* L./**LP/1 site**
51. *Saxifraga bifora* All./**SB/1 site**
52. *Schoenoplectus mucronatus* (L.) Palla/**SM/1 site**
53. *Schoenoplectus triqueter* (L.) Palla/**ST/1 site**
54. *Scutellaria alpina* L./**SL/1 site**
55. *Thalictrum alpinum* L./**TA/1 site**

Other species listed in appendix II of EU Habitat directive, endangered and occurring in Trentino (1)

*** 56. *Cypripedium calceolus* L./**CC /10 sites**

Trentino narrow endemic species (17)

57. *Campanula petraea* L. /**CE/9 sites**
58. *Carex baldensis* L./**CB/10 sites**
59. *Centaurea rhaetica* Moritzi /**CH/10 sites**
60. *Draba dolomitica* Buttler/**DD/10 sites**
61. *Erysimum aurantiacum* Leyb./**EA/8 sites**
62. *Euphorbia variabilis* Cesati/**EV/10 sites**
63. *Festuca austrodolomitica* Pils & Prosser/**FA/10 sites**
64. *Hypochoeris facchiniana* Ambrosi/**HF/8 sites**
65. *Laserpitium nitidum* Zanted./**LN/10 sites**
66. *Moehringia glaucovirens* Bertol./**MG/10 sites**
67. *Primula spectabilis* Tratt./**PS/10 sites**
68. *Primula tyrolensis* Schott/**PT/10 sites**
69. *Pulmonaria vallarsae* A. Kerner/**PV/10 sites**
70. *Rhinanthus songeonii* Chab./**RS/10 sites**
71. *Saxifraga depressa* Sternb./**SD/10 sites**
72. *Saxifraga hostii* Tausch subsp. *rhaetica* (Kerner) Br.-Bl./**SH/4 sites**
73. *Scabiosa vestina* Facch. ex Koch/**SS/10 sites**

Appendix 3. Details of the seed collecting activity.
For site codes see Appendix 2. Collectors codes are as follow:
AB Alessandra Bonazza; AC Alessandro Cavagna;
CB Costantino Bonomi; FP Filippo Prosser.

Date	grid	destination	Sites where seeds were collected	Sites where seeds could not be collected because not yet mature or plants sterile	Sites where target species could not be found	collector/s	ascent on foot	Km trevelled	working hours
18-giu-02	0130/3	Tremalzo		OS3(pre-visit)		GP CB AC	50	120	8
18-giu-02	9731/3	Rif. Casinei - Val Brenta		CC1(early)	OS2	GP CB AC	437	73	4
21-giu-02	0230/1	Campione del Garda	DR6 DR7	DR5(late)		CB AC AB	140	52	11
23-lug-02	0133/1	Passo Coe			HC1	AC	0	10	3
24-lug-02	0130/3	Tremalzo	OS3 VD6	CH4(early) LN6(early) PS7(early)		CB AB	272	160	10
25-lug-02	9832/1	Terlago	VE1			CB AB	0	24	6
25-lug-02	0032/1	Cei		PV5(late)	GP4, OE1	AC	0	20	3
25-lug-02	0132/1	S. Barbara, Cengio Alto	GP1 GP11			AC	350	30	5
26-lug-02	0231/3	Cima delle Pozzette	CK5	CK3(late)	KP4	CB AB	380	121	13
26-lug-02	0033/2	Passo Coe -Passo Vezzena			CD2, CD3, HC1	AC	0	64	8
27-lug-02	9732/4	Mt. Cornello	DA3			CB AB	633	50	7
27-lug-02	9732/4	Torre di Visione	DA2			CB AB	415	31	4
28-lug-02	0129/4	Bocca di lorina		PC6(early) AT2(early) CH3(early) RS3(early)	MG9, KV3, CB5(da escludere)	CB	541	113	9
30-lug-02	9732/2	cima d'Arza	DA1			CB AB	586	69	7
31-lug-02	0129/4	Val Lorina M.ga Valesina M. Caplone	CH3 AT2 CB11 RS3 VD11			CB AB	769	155	13
31-lug-02	0133/1	Martinella		KP (early)		AC	300	0	3
01-ago-02	9536/1	Torbiera di Talpian Val di Fassa	JA2 TA1	JA1(sterile)		CB AB	444	212	10
02-ago-02	0131/2	Carpaneda, Gaz Verde			GP2, GP7	AC	450	58	8
03-ago-02	0129/4	M. Tombea - Val di Ledro	Car Broke down			CB	0	180	10
04-ago-02	0129/2	Ampola Alla Tolla	AT1 SA2 MG6			CB	50	50	4
04-ago-02	0130/3	Tremalzo - Corno della Marogna	DP9		SA4	CB	250	38	2
07-ago-02	0129/2	Malga Stigolo, Tiarno		DP3(late)		CB	230	64	8
07-ago-02	0130/3	Corno della Marogna, Tremalzo	DP9		SA4	CB	0	50	4
07-ago-02	0033/2	Passo Coe -Passo Vezzena	CD2	CD1(late)		AC	0	54	8
08-ago-02	0131/3	Monte Altissimo di Nago	CK1	ST2(late)		CB AB	600	112	10
08-ago-02	0033/1	Scanuppia	SO1		GP3	AC	1500	42	11
10-ago-02	0129/3	Bondone di storo	MG8 SA7	EV7(late)	AT8 RS2	CB AB	0	178	11
12-ago-02	0033/3	Vigolana		PS5(early)	SO3	AC	850	13	12

Date	grid	destination	Sites where seeds were collected	Sites where seeds could not be collected because not yet mature or plants sterile	Sites where target species could not be found	collector/s	ascent on foot	Km trevelled	working hours
13-ago-02	9932/2	Roncafort	SM1	LP1(late)		AC	0	68	8
13-ago-02	9932/2	Doss Trento	EH1			CB AC AB	70	10	4
14-ago-02	0129/4	Monte Tombea - Val di Ledro	VD8 AT11 AT12 MG11 PS11 CH11			CB AC AB	550	207	16
15-ago-02	0029/3	Valle Aperta Malga Bondolo (Condino)	PG3 SH2 SV10	CR4(early)		CB AB	176	221	14
17-ago-02	0229/1	Mte Cingla - Storo	TS2	GP6(early)		CB AB	135	258	14
17-ago-02	0129/4	Mte Tombea - Val di Ledro	VD8 CH12 PS11			CB AB	130	0	
20-ago-02	9535/4	Roda di Vael - Val di Fassa	RA7	CM7(early)		CB AC	592	227	13
21-ago-02	9836/4	Val Noana - Primiero			PT7	CB AB	20	229	10
21-ago-02	9737/3	Val Canali - Primiero	PT3	CM10(early)		CB AB	40	0	
21-ago-02	9935/4	Grigno	AT6		OE3	AC	200	132	10
22-ago-02	0332/2	Campobrun - Lessini Trentini	CC2		AL1	CB AC AB	560	312	14
22-ago-02	0232/4	Campobrun - Lessini Trentini	PS13	PR3(few)	AL2	CB AC AB			
23-ago-02	9731/3	Val Brenta		CC1(early)		AB	0	130	5
24-ago-02	0129/4	Valle del Comune - Lorina		SA5(few) DP7(late) SS8(few)		CB AB	890	149	12
26-ago-02	0232/4	Cornetto	PC5			AC	850	13	7
27-ago-02	9928/4	Passo di Campo Val Daone	SV2 PD4			CB AB	521	234	13
28-ago-02	0132/4	Roite			PS6	AC	600	76	8
28-ago-02	9731/2	Cima Uomo - Brenta			DD10	CB AB	730	123	12
29-ago-02	9933/1	Lago Pudro Madrano	LL4		CD5	CB AB AC FP	0	49	7
30-ago-02	0332/2	Passo Malera, Monte Zevola, Passo tre Croci	AL1 AL2 PR2			AC	900	242	15
31-ago-02	9636/4	Val Venegiotta, passo Rolle	JA3 JA4 JA5		DD3 PT1	CB AB	315	249	13
31-ago-02	0332/2	Castel Gaibana (Ala)	AL5			FP	698		
02-set-02	0030/4	Malga Tratt, Mazza di Pichea, Val di Concei	HF7	ST5(late) CH7(late)	VD5	CB AB	410	153	13
02-set-02	0132/4	Monte Buso	PS6			AC	50	54	6
07-set-02	0029/3	Passo del Bruffione - Valle Aperta (Condino)	PG1 AN1 AR1			CB AB	520	200	13
07-set-02	0332/2	Monte Zevola, Cima Campetto, Passo Porta	AL1 PR1 PR2			AC	1100	181	15
08-set-02	0232/4	Guglie del Fumante - Vallarsa	PR5			CB AC	519	89	8
09-set-02	9536/2	Col del Cuc - Passo Pordoi	DG1			CB AB	193	240	11
09-set-02	0231/3	Monte Baldo	PS8			AC		52	7
10-set-02	9934/4	Sentiero della Caldiera - M. Ortigara	PT5	CC9(early) PS9(few, sterile)		CB AB	730	105	10
10-set-02	0331/3	Rivalta, Spiazzi, Ceraino, Ca Verde	CE5	CE4(early) CE6(early)	CE2	AC	500	151	11

Date	grid	destination	Sites where seeds were collected	Sites where seeds could not be collected because not yet mature or plants sterile	Sites where target species could not be found	collector/s	ascent on foot	Km trevelled	working hours
11-set-02	9932/2	Roncafort, Doss Trento	EH1			CB AB AC	70	10	5
12-set-02	9830/2	Doss del Sabion Pinzolo	PD10			CB AB	510	157	11
13-set-02	9932/2	Doss Trento	EH1			CB AC	70	10	4
14-set-02	9730/4	Lago Ritorto - Campiglio	PD6			CB AB	71	181	11
14-set-02	9830/2	Val d'Agola - Campiglio	SL1 AN11			CB AB	110	0	
15-set-02	9629/4	Monte Palù -Vermiglio Alta Val di Sole			DG2	CB AB	1142	220	14
17-set-02	9737/3	Passo delle Lede - Pale di S. Martino	RA11 PT10 SF9			CB AB	1269	234	15
23-set-02	0129/1	Monte Pissalat - Giudicarie	PG4 SH1			CB AB	228	229	12
25-set-02	0229/1	Monte Cingla - Storo	LN11 TS2			CB AB	225	192	12
26-set-02	0129/4	Monte Caplone - Val di Ledro	SE11			CB AB	349	174	11
28-set-02	0129/2	Cima del Visì Val di Ledro			SE9	CB AB	475	140	11
29-set-02	0130/3	Corno della Marogna, Tremalzo	SE8			CB	90	86	5
30-set-02	9929/4	Monte Benna			SE1	CB AB	314	191	12
02-ott-02	0030/2	Fiavè	LL2			CB AC	0	50	5
02-ott-02	9930/4	Passo del Durone	LL1			CB AC	0	61	5
03-ott-02	9934/4	Grigno, Caldiera	CC9 PS9			AC	500	149	10
04-ott-02	0132/4	Bocchetta Foxi, Lancia			CC10	AC	400	76	10
05-ott-02	0130/1	Corno Spezzato			LN7 SE4	CB AB	255	170	14
07-ott-02	9436/4	Val Lasties, Fedaia, Val Udai, Soraga	CC5 CM8 CM9			AC	300	200	12
08-ott-02	9436/4	Val Lasties, Fedaia, Val Udai, Soraga	AN8 AN9			AC	300	143	12
08-ott-02	9532/2	Fondo Alta Anaunia	AN7			CB AB	53	177	8
08-ott-02	9933/4	Alberè di Tenna			LL5	CB AB	7	0	2
09-ott-02	0029/3	M. Remà Valle Aperta	CR4 PG3			CB AB	176	215	11
10-ott-02	9535/4	Roda di Vael - Val di Fassa	CM7 RA7			CB AC	592	211	10
13-ott-02	9731/3	Val Brenta	CC1			CB AB	0	150	5
14-ott-02	9929/3	Bassa Val Daone	CR1			CB AB	593	143	10
14-ott-02	9736/2	Cimon della Pala		CM4(early)		AC	500	266	11
15-ott-02	0032/1	Val Mana Tre Cime del Bondone			AN2	CB AB	414	76	8
15-ott-02	0332/2	Revolto, Ca' Verde	CC2	CE6(early)		AC	150	266	9
16-ott-02	9732/2	Vigo di Ton	TH1			CB AB	0	64	3
18-ott-02	0130/3	Corno della Marogna, Tremalzo	SS9			CB AB	90	176	5
07-nov-02	9935/4	Grigno	PC4			AC		132	5
10-nov-02	9736/2	Cimon della Pala	CM4			AC	500	266	12
12-nov-02	9636/4	Castellazzo	CM5			AC	300	273	10
20-nov-02	0331/3	Ca' Verde, Spiazzi	CE4 CE6			AC	50	134	7
		TOTAL					**31349**	**11519**	**808**

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A Plant Recovery Programme in the 'Conservatoire Botanique National de Brest' (France)

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Abstract. *– A programme for the rescue of critically endangered plants worldwide has been initiated in Brest, France. Its purpose is to create a database for these plants, their history, their situation and the progress made for their rescue. This initiative is in close relation with international bodies of conservation. The main advantage should be to establish a permanent update about these plants and the issues for their recovery. As a first step, we are working upon the critically endangered plants of Europe.*

Key words: *extinction, recovery, rescue.*

Introduction

In 1975, we have created the 'Conservatoire Botanique National de Brest', a structure devoted to the rescue of endangered plants. Now, it includes a botanic garden with a relatively small number of taxa, 3,500, but with a large proportion of threatened species, 1,500 of which 450 are critically endangered or extinct in the wild and rarely cultivated.

Currently, a new specialized project is in progress in Brest. This project has been presented under the name of 'International Secretariat for the Rescue of Plants' (Lesouëf 2001) and is now entitled: 'Plant Recovery Programme'.

How many species are threatened?

The *1997 Red List of IUCN* records about 35,000 taxa as threatened. The true number is certainly higher, because many tropical species-rich countries have little contributed to this inventory.

Much larger figures have been given. We follow David Bramwell (2002, 2003): out of the 400,000 species of plants in the world, 100,000 would be threatened.

For the critically endangered or extinct plants, we have combined several methods and we have estimated that roughly 5% of the world flora would be critically endangered or extinct. These species are the long-term target for the Plant Recovery Programme.

The methods of conservation

Most of the living plants do not need specific actions. They will survive and evolve in their habitats. For the less threatened species, adequate *in situ* conservation actions, in reserves and other protected areas, are sufficient.

It is frequent that a critically endangered species is under an immediate risk of disappearing although it may be legally protected and growing in protected areas. Specific management or recovery measures are required to improve the situation and to remove the species from the critical situation.

E. Robbrecht & A. Bogaerts (eds.) EuroGard III. Papers from the *Third European Botanic Gardens Congress* and the *Second European Botanic Gardens Education Congress* (BEDUCO II).

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***Centaurium favargeri* Zeltner**	**Category**	**Cult.**	**Distribution**	**Cultivation**
	EW Criteria	1	- FRANCE (EW) - FRANCE : Languedoc Roussillon : Aude (11) (EX) - FRANCE : Languedoc Roussillon : Hérault (34) (EX) - FRANCE : PACA : Htes Alpes (05) (EW) - FRANCE : PACA : Bouches du Rhône (13) (EX) - FRANCE : PACA : Vaucluse (84) (EW) - GEORGIA (EX)	BREST: Conservatoire Botanique National (2003) GAP: Conservatoire Botanique National Alpin (2003) KEW: Royal Botanic Gardens NEUCHATEL: Jardin Botanique de Neuchatel (1976)
GENTIANACEAE 2003				4 gardens
Commentary This small annual has been recorded from a relatively large area: 11 localities in the South of France and also in Georgia. It is a plant of open habitats, clearings of ripisylves, riversides, etc. Most of records are old. The last record is mentioned in 1990 at Valserres (Hautes-Alpes), with two populations totaling about 3000 specimens. These populations close together have been washed out by a flooding of the river Durance, which has carried away the substrate. Fortunately seeds of this population as well as the type population (Cheval-Blanc, Vaucluse) have been collected before extinction, so that the species is maintained in cultivation. References of this species in Portugal and Spain are erroneous.				

For these species, *ex situ* conservation is extremely important. Sometimes, the survival of a species is absolutely dependant on cultivation considered as part of a recovery programme.

Cultivation is important in several ways. First, we can produce large quantities of living material for reintroduction or to understand some aspects of the biology of a species. Second, cultivation and especially seed-banking may be the ultimate barrier against a definitive extinction. It may be noted that modern management practices use an array of measures between pure *in situ* measures such as reserves and pure *ex situ* measures such as cultivation and seed-banking.

Intermediate practices are for example: weeding of selected reserves such as in oceanic islands invaded by exotics or in continental areas, in bogs, shingles, etc. everywhere when it is necessary to maintain open habitats, reintroductions, strengthening of populations, etc.

The line between *in situ* and *ex situ* activities are not clear cut. Now, it is admitted that an integrated *in situ / ex situ* approach is the best way.

The rescue of endangered species by cultivation is a relatively new concept and we have to deplore the definitive extinction of numerous species in spite of them having cultivated but being also ultimately lost in cultivation. The monotypic genera *Astiria* (Mauritius), *Benedictella* (Morocco), *Mathieua* (Peru), *Streblorrhiza* (Norfolk island), etc. are in this case, as well as the European taxa *Herniaria ciliolata* subsp. *subciliata* (Jersey), *Saxifraga oppositifolia* subsp. *amphibia* (Germany & Switzerland), *Althaea kragujevacensis* (Serbia), *Vicia dennesiana* (Azores), *Viola cryana* (France), etc.

These species would not have been difficult to grow, but they were lost by lack of care and lack of coordination. Conversely, an increasing number of critically endangered plants are now rescued and have recovered through a combination of *in situ* and *ex situ* measures.

Principles of the 'Plant Recovery Programme'

Selection of the species. Contrary to the BGCI, The European Plant Conservation Strategy and the IUCN / Species Survival Commission which take into account the whole of the threatened species, the scope of this programme is limited to the small proportion of the flora which is critically endangered or extinct. It is a very narrow and specialized objective. In fact, it is a close up on these species to be carried out in close relation with the above mentioned bodies. We hope it will be a useful contribution towards the identification of these species and their rescue.

Protection	**Threats**	**Recov.**
☐ **Bern Conv.** ☐ **Habitats Dir.**	1.4 - Infrastructure development 1.4.2 - Human settlement 7.2 - Flooding 9.9 - Restricted range 12 – Unknown	1

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Table 1. Data sheet for *Centaurium favargeri*.

Cult.: Rating of cultivation – 0 = not in cultivation, 1 = cultivated between 1& 5 gardens, 2 = cultivated between 6 & 20 gardens, 3 = cultivated in more than 20 gardens, 4 = cultivated without precise site
Recov.: Rating of Recovery Program 0 = No Recovery Program, 1 = initiated, 2 = in progress, 3 = achieved, 4 = unknown
Threats: According to the IUCN Assessment

The database includes following information for each plant (see table 1 for further details): name of the plant; category with criteria of the IUCN; frequency in cultivation; geographic distribution; estimate of the degree of completion of a recovery programme; a synthesis relating the salient facts about species, its history, the actions already led or in progress and the main threats; references.

'Sites of cultivation' includes Botanic Gardens, private or public collections, commercial nurseries and seeds producers. If less than six, the sites of cultivation are mentioned with their last known year of holding the plant. In any case, all the sites participating to a recovery programme are mentioned. Details on the number of specimens, origin, seed-banking, genetic diversity when available are kept in the data base, but not published.

Advantages

1. The main interest of this programme will be to maintain an up to date information on each critically endangered or extinct plant. It will be also a place where the participants to the conservation will have the possibility to publicize their works and their results. People searching for an issue relating to their plants would consult the site to find information about plants in similar situations.
2. This programme could also allow to detect the gaps in the rescue of these plants and to propose urgent rescue measures to the local conservationists.
3. As a first initial result several critically endangered species which do not or rarely appear in botanic gardens have been detected in other cultivated sites: *Isoetes malinverniana* which is relatively widespread among aquariophilists and the almost extinct *Myosotis azorica* which is not in the botanic gardens but which is sporadically cultivated by amateurs.

The extinct *Vicia fulgens* from Algeria has been detected in cultivation in three gardens, but these data are 30 years old and the plant is possibly extinct also in cultivation.

Development. At present we are working for the Council of Europe and the European Topic Centre for Nature and Biodiversity to create a database for the most threatened plants of Europe. We take into account only the species critically endangered or extinct in Europe, excluding the plants which are widespread elsewhere. The number of taxa would be around 500. A well-known fact is already being confirmed: the northern countries have very few endemics and they are of a low taxonomic rank. Few of them are critically endangered. Even within the Mediterranean peninsulas, the southern regions are more rich than the northern: Andalusia, Puglia, Calabria, Southern Greece and the Mediterranean islands. In Eastern Europe, Southern Ukraine and especially the Crimea and the extreme South of Russia have also the greatest proportion of endemics. Our work also includes Macaronesia, as well as Cyprus. Apart from our European work, we are also entering data recording about New Caledonia, oceanic islands, American bamboos, etc. in collaboration with specialists.

A practical use of these data. In New Caledonia *Pittosporum tanianum* is a tree limited to three specimens and under serious threat by overgrazing by introduced deer. This species is doomed unless immediate steps are taken towards a recovery programme. Already, people of New Caledonia have tried to cultivate it. The people engaged in the conservation of this plant have been interested to learn that for another *Pittosporum* in a similar situation, the Mauritian Wildlife Foundation in collaboration with the Forestry Service of Mauritius has realized the rescue of the extremely rare *Pittosporum balfourii*, endemic of Rodrigues and which was reduced to 20 specimens in the wild. They have propagated the tree, reintroduced 8,000 specimens in the wild after the control of the invasive species. Now, the native forest of Grande Montagne Reserve of Rodrigues is in an excellent process of recovery. One can see the interest to cross these data.

Critically endangered conifers. Conifers are a well identified group thanks to the activities of the Group of specialists "Conifers" of the IUCN / Species Survival Commission which have produce very accurate figures. Among the 800 conifers, 200 are threatened of which 21 are critically endangered. We have treated this number with our method of analysis of the frequency in cultivation. At least seven out of 21 are cultivated in more than six gardens, some of them being cultivated in quantities reaching millions of specimens such as for *Metasequoia*. Seven are cultivated in one to five gardens and seven are not at all cultivated. Three of them are Caribbean *Juniperus* and it is clear that an urgent rescue programme is needed if these trees have to survive. Seven out of 800 conifers which are critically endangered are not yet in cultivation ; this constitutes a very manageable figure which is not at all beyond the reach of the scientific and conservationist world community.

Conclusion

We strongly urge the participants of this Congress to contribute to this programme, especially for its European part. It would be very useful for the community of botanical gardens to know more about what has been done in similar situations of habitats, or under similar climates, for similar type of plants and in similar cases of extreme risks, etc.

Acknowledgments. – We would like to thank Peter Wyse Jackson, director of the B.G.C.I., and Wendy Strahm, officer in charge of the Species Survival Commission of I.U.C.N., for their assistance in the first years of the Plant Recovery Programme.

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Seed bank of threatened plants in the 'Conservatoire Botanique National de Brest' (France)

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Abstract. *– The Conservatoire of Brest hosts an important seed bank, which is probably the most practical technique for* ex situ *conservation of plants. Seeds are the most useful living material for a long-term conservation due to their small size and their viability. The seed bank of the Conservatoire concentrates upon threatened species worldwide, and so is one of the most important places for the conservation of endangered plants. Examples of results are given, such as the successful reintroduction of* Normannia triphylla *in Madeira.*

Introduction. For now over twenty years, the 'Conservatoire Botanique National de Brest' (Brittany, France) has been working on the safeguard of threatened species from all over the world (see Lesouëf 2004). It hosts one of the most important collection of threatened plants, comprising more than 1500 species. Its *ex situ* conservation is largely involved with species on the brink of extinction. Beside cultivated plants in glasshouses or in the garden, the Conservatoire keeps an important seed bank, which during the past 27 years has proved to be a valuable tool for the preservation of threatened species.

Why a seed bank? A seed bank is probably the most practical technique for *ex situ* conservation of plants.
- Seeds are the most convenient form of living material for long-term conservation due to their small size and their viability.
- In small spaces, huge quantities of species can be maintained at low cost.
- The technique allows preserving the genetic variability of the species.
- A seed bank represents a reserve for any future reintroduction as well as for exchange between scientific institutions.

Seed bank organisation in the Conservatoire. Seeds have been stored since 1976 in freezers at -20°C; four freezers are needed to keep 1,730 species and more than 3,500 batches. Among the species in the seed bank 73 are extinct (EW), 215 critically endangered (CR), 335 endangered (EN), 447 vulnerable (VU); 215 protected species from Brittany are also represented.

The process of seed banking includes differents stages: collecting in the wild or in cultivation, cleaning and counting, drying in a desiccator during 2-3 months, testing the viability of seeds before storage, and storing in freezers, sheltered from light, humidity and air. Further tests of viability have to be done at various intervals (2.5 or 10 years). According to the test results, we decide whether renewal of the stock is necessary or not.

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Some results

Normania triphylla (Lowe) Lowe – A member of the Solanaceae only growing in Madeira and which had been considered extinct for a century. Manuel de Nobrega discovered one plant in 1991, seeds were collected from it and sown in Funchal, but the dry climate did not allow to multiply the species in the site. A sample of 24 seeds was sent to the Conservatoire Botanique in 1994 and the species now thrives in Brest.

Seeds are regularly collected, one part is sown and the other part is tested and stored in freezers. Currently, we have 14,000 seeds in storage that are regularly tested. Seeds were sent to Funchal, allowing reintroduction of a hundred plants in Madeira in 1998.

Lysimachia minoricensis Rodr. – A plant of the Baleares, extinct in nature. In 1977, the Conservatoire Botanique has obtained seeds from the Botanic Garden of Berlin-Dahlem. We have sowed the seeds to obtain a massive stock. Today we have more than 200,000 seeds in storage since 26 years and there is no loss of viability.

Eryngium viviparum J.Gay – Vulnerable plant (Olivier & al. 1995: 187). There is only one locality in Brittany which benefits from an *in situ* programme of conservation. We regularly collect seeds in nature, and at present we study the seeds' behaviour under storage conditions.

Centaurium favargeri Zeltner – An endemic of France, extinct in the world (Olivier & al. 1995: 128). Seeds have been stored since 1982, and 75% of seeds are viable after fifteen years of storage.

Polygala antillensis Chodat – Critically endangered in Martinique. We have 1,800 seeds stored since 1983, and 100% of viable seeds after 10 years of storage.

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Threatened plant collections in 'Alexandru Borza' Botanical Garden (Romania) for research and conservation purposes

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Abstract. – *This paper surveys the threatened plants conservation activities of the "Alexandru Borza" Botanical Garden (Cluj-Napoca) between 1992 and 2003, viz. (1) chorological, phytocoenological, population studies, classical and in vitro multiplication as well as acclimation studies on some threatened taxa from Romania; (2) the setting-up of a new sector, intended for endemic and threatened species from Romania. Two new localities were identified for* Aquilegia nigricans *subsp.* subscaposa *and* Centaurea reichenbachii, *respectively.* Aquilegia nigricans *subsp.* nigricans, Centaurea reichenbachii, Dianthus petraeus *subsp.* orbelicus *and* Nigritella nigra *subsp.* rubra *are newly reported from two associations. The analyzed taxa have a high competitive capacity, and were positively correlated with numerous species from the coenosis where they develop. The analysis of the aggregation tendency revealed that the type of the phytocoenosis and the adaptative strategy adopted by each taxon influence the presence and the extent of aggregation. The limited surfaces occupied by the populations of the analyzed taxa justify the maintenance of their current status on the national red lists. Depending on the species and age of the seeds, under laboratory conditions, the seed germination capacity was always positively influenced by the constantly applied low temperature and the number of exposure hours. Significant differences also appear between the germination capacities of the different populations belonging to the same taxon (e.g.* Centaurea reichenbachii*). Significant results were obtained by in vitro multiplication experiments, in case of cultures initiated through seed germination. Under* ex situ *conditions, the viability percentage of individuals obtained from seed depends on the species and culture conditions. These data as well as the plant material collected in the field, allowed to set up the collections intended to the sector of Endemic, Rare and Endangered plants from Romania.*

Key words: *threatened taxa, phytocoenological work, population studies, seed germination,* in vitro *multiplication, acclimation, database, threatened plant collections, conservation.*

Introduction

The majority of the European botanical gardens are affiliated to universities, thus acquiring the attributes of research centres that contribute to the conservation of phytodiversity, in particular by the inclusion in their collections of species threatened at national or international level and by their involvement in

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processes of *in situ* conservation, This is also the case of the Botanical Garden from Cluj-Napoca, founded in 1920, whose history is closely connected to that of the Cluj University and to the name and activity of the eminent professor Alexandru Borza.

Although it occupies a relatively small area – 14 ha – the undulating surface offers various microclimates, which has allowed the culture of a large number of taxa from all over the world. Its collections shelter approximately 9,800 taxa, cultivated in greenhouses or in open air, as part of the six existing sectors: the Ornamental (1,380), the Phytogeographical (4,314), the Systematic (3 350), the Economic (220), the Nursery (475) and the sector of Endemic, Rare and Endangered plants from Romania (60). A botanical Museum (with approximately 7,000 exhibits), a Herbarium (the most comprehensive one from Romania, more than 660,000 sheets and 325 holotypes) and an Office for Plant Material Exchange are further elements of the Botanical Garden (Cristea & al. 1997; Micle & al. 2002).

Through its collections and activities, the institute assists in the process of scientific research, being an important centre for the protection and conservation of phytodiversity.

The present paper synthesizes the concerns of the researchers of the "Alexandru Borza" Botanical Garden regarding conservation activities, with a particular focus on threatened plant collections. The setting-up of viable collections required complex information on the biology, ecology and *in* and *ex situ* behaviour of the threatened species.

In this context, the studies performed (in the period 1992-2003) on some taxa with special phytogeographical importance in the Romanian flora, resulted in: (1) the chorological re-examination of the studied taxa; (2) the carrying out of phytocoenological work, population studies, classical and *in vitro* multiplication experiments and acclimation studies; (3) the setting-up of the Endemic, Rare and Endangered plants from Romania sector; (4) the revision of the protection status of target taxa; and (5) the elaboration of databases to be used for both the preparation of the red list and book of plant species and for regional and national programmes for the evaluation, monitoring and conservation of phytodiversity. The results of the performed studies supplied complex information concerning the biology and *in* and *ex* situ behaviour of the threatened species. These data and the plant material collected in the field allowed to conceive and organize the special collections for the sector of Endemic, Rare and Endangered plants from Romania.

Study area, material and methods

The geographical areas taken into consideration for the phytocenological work and population studies, covers the mountain group Gilău-Muntele Mare, on the Estern side of the Western Transylvanian Carphatians (Romania), Suatu (e.g. *Astragalus peterfii*) and Fânaþele Clujului natural reserves (e.g. *Adonis volgensis* and *Bulbocodium versicolor*). The species nomenclature follows *Flora Europaea* (Tutin & al. 1964-1980).

Phytocoenological and population studies, as well as classical and *in vitro* multiplication and acclimation studies were performed on the threatened and/or endemic taxa: *Adonis volgensis* (RRL), *Astragalus peterfii* (End., RLTP, GTPE, BC, RRL), *Aquilegia nigricans* subsp. *subscaposa* (End., GTPE, DirHab, RRL), *Bulbocodium versicolor* (RRL), *Centaurea reichenbachii* (RRL), *Dianthus petraeus* subsp. *orbelicus* (RRL), *D. spiculifolius* (GTPE, RRL), *Nigritella nigra* subsp. *rubra* (CITES, RRL) and *Saponaria bellidifolia* (RRL). The researches performed were based on methodologies specific for different investigation lines. [Abbreviations: End., endemic species; RRL, Romanian red lists, RLTP, IUCN-Red List of Threatened Plants; GTPE, Globally threatened plants in Europe; BC, Bern Convention; CITES, CITES List.]

Phytocoenological studies were carried out based on phytosociological releves (performed according to the central European method, Braun-Blanquet 1964), the assignment to associations being done by both classical methods, based on characteristic species, and modern methods, using dendrograms (based on the Sørensen index, *average-linkage* algorithm, Podani 1993).

Using the correlations with species existing in the phytocoenotic environment, calculated by means of the Spearman coefficient, the constellations allowing to show the eco-coenotic nucleus of each analyzed taxon were elaborated.

For the analysis of spatial distribution, representative sample surfaces were selected (25 m^2), in which the position of each individual was determined by measuring the distance between these individuals and the borders of the sample surface. The aggregation tendency was estimated based on spatial distribution of the target taxa.

In the case of the *Astragalus peterfii* species, sampling along one transect (using a 2x2 m square frame) was performed, which covered an area of 188 m^2 (2x94 m). This was located so as to intersect the various plant communities in which a decrease in the number and density of individuals was found. Within each 2x2 m square, phytosociological relevees of the cormophyte species and the positioning of the species individuals were performed, with the lower left corner of the square as a reference. Subsequently, depending on the density found in *Astragalus peterfii* individuals, into two areas it was subdivided the transect. The areas were compared from the point of view of the floristic composition and the species with discriminatory value were evidenced in the two areas.

The observations regarding seed germination were performed on ten year old groups and on fresh seeds (collected from various population groups). These were submitted to low temperatures of 0° C, applied constantly and in alternation with 20° C temperatures (12 h/12 h). These experiments were performed on *Centaurea reichenbachii* and *Dianthus petraeus* subsp. *orbelicus* seeds.

In order to improve the concerns focused on the conservation of these species, researches were completed by experiments regarding their *in vitro* multiplication capacity. For this purpose, cultures were initiated starting from apical, nodal explants, flower buds and seeds, on basic Murashige-Skoog medium. The culture media contained macro- and microelements, supplemented with vitamins, saccharose and agar. The phytohormones used were ANA (naphtaleneacetic acid) and BA (6-benyzlaminopurine). The sterilization of plant material was done with Domestos 100%, for 15 minutes. The culture were mainteined at a temperature of 25 ± 2^{0}, at a light intensity of 87 ìmol/m^2/s and a photoperiod of 16 h light / 8 h dark (Cristea Victoria & al. 2002).

The acclimation studies, carried out over two years (2002-2003), were performed on the living material taken from the field and individuals obtaine from collected seeds (e.g. *Aquilegia nigricans* subsp. *subscaposa* and *Centaurea reichenbachii*). These individuals were cultivated under different conditions: 1 - in manure (in pots); 2 - in the nursery; in garden soil, in beds, intended for families (Systematic sector); 3 - on calcareous rocks in the sector of Endemic, Rare and Endangered Plants from Romania. The observations were performed on 90 plantlets, 30 for each group, in the case of *Centaurea reichebachii*, and on 60 plantlets, 20 for each group for *Aquilegia nigricans* subsp. *subscaposa*.

For information processing and database creation, the software characteristic for and applicable to the aims of the study was used Syn-Tax 5.0 and SAS Stat 7.0-Online Doc, MS - Office (Podani 1993; SAS Institute 2000).

The creation of the collections intended for the sector of Endemic, Rare and Endangered plants from Romania aimed to ensure a certain level of genetic variability, the biological material used being taken from representative and aleatory samples belonging to different populations. The seed collected in the field were showed and kept in wood pots (in Systematic sector nursery) and planted in the new sector after one year. The living material collected from the field was, also mantained a year in the nursery and after that was transplantated.

Results and discussion

Research on some phytogeographically important Romanian taxa

Chorological studies. In order to evaluate the presence of taxa, the literature data were checked in the field. The taxa included in the study were found in the mentioned localities, except for *Aquilegiei nigricans* subsp. *subscaposa*, which was only reported in two localities of the four mentioned in the literature (Hentea & al 2002). In contrast, following the researches performed, the presence of the subspecies was reported in a new locality, situated in the same basin as one of the localities from which it disappeared (Hentea & al. 2002).

The disappearance of the taxon from these localities, situated at a lower altitude, was probably caused by harvesting for its ornamental value.

In the case of *Centaurea reichenbachii* (species with a distribution limited to the Western Transylvanian Carphatians), the identification of a new locality should be reported. This extension of the distribution is extremely important considering that populations are represented in the territory by a reduced number of individuals.

Phytocoenological studies. In parallel with the evaluation of the presence of taxa, the eco-coenotic environment in which the taxa studied reach an optimum development, was analyzed. The researches performed reported the presence of the taxa *Aqulegia nigricans* subsp. *subscaposa*, *Centaurea reichenbachii*, *Dianthus petraeus* subsp. *orbelicus*, and *Nigritella nigra* subsp. *rubra* in two associations other than those mentioned in the literature.

For most taxa, based on the analysis of the correlations between the studied taxa and the species existing in the phytocoenotic environment, it was shown that the large number of the positive correlations recorded (e.g. *Aquilegia nigricans* subsp. *subscaposa*, fig. 1) reveals an increased level of competitive capacity and coenotic cohesion, so a complex structure of phytocoenoses.

For *Centaurea reichenbachii* and *Dianthus petraeus* subsp. *orbelicus*, the negative correlations shown (fig. 2) demonstrate the lower coenotic cohesion, these taxa growing well only in rocky calcareous communities.

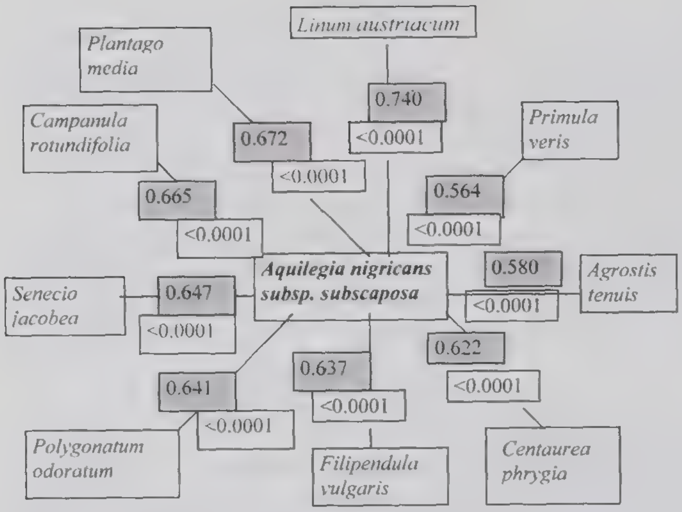

Figure 1. Main species of the coenotic environment correlated with Aquilegia nigricans *subsp.* subscaposa *(only species with correlations higher than 0.550 and a probability less than 0.0001 were represented).*

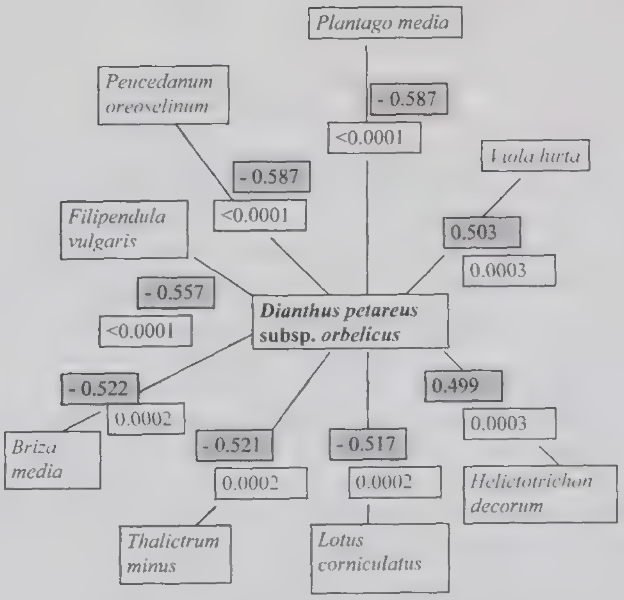

Figure 2. Main species of the coenotic environment correlated with Dianthus petraeus *subsp.* orbelicus *(only species with correlations higher than 0.499 and a probability less than 0.0003 were represented).*

Population studies. These studies, correlated with the phytocoenological ones, are necessary in order to establish the strategies for the conservation and repopulation of threatened species.

The population studies, carried out for the first time in the case of the analyzed taxa, aimed to evaluate the spatial distribution of taxa in characteristic plant associations, and, based on it, to test the aggregation tendency, to analyse density and to establish the species with discriminatory value.

The aggregation tendency showed by the studied taxa depends on the type of phytocoenosis and is determined by the adaptative strategy adopted by the taxa. Depending on species, it is probably due to an increased dispersion capacity of seeds (e.g. *Aquilegia nigricans* subsp. *subscaposa*, fig. 3 & 4), to an increased number of seeds (*Saponaria bellidifolia*), to the multiplication capacity (e.g. *Bulbocodium versicolor, Astragalus peterfii*) and vegetative regeneration (e.g. *Dianthus petraeus* subsp. *orbelicus*).

In the case of *Centaurea reichenbachii*, the reduced number of individuals by which this taxon was represented in the analyzed sample surfaces did not allow the statistical processing of population parameters.

The analysis of interrelations between observed spatial distribution of the *Astragalus peterfii* individuals and the floristic composition of the nearest plant communities, revealed the existence of discriminant species *Artemisia campestris* and *Carex humulis* (fig. 5) (Şuteu & al. 2003).

Protection status. The observations and studies performed showed that the analyzed taxa, except *Centaurea reichenbachii* and *Saponaria bellidifolia*, develop populations represented by an increased number of individuals, which occur on small surfaces in their distribution area, punctiform in most cases (e.g. *Adonis volgensis, Astragalus peterfii, Aquilegia nigricans* subsp. *subscaposa, Nigritella nigra* subsp. *rubra*).

The results of phytocoenological and population studies shows that is necessary to maintain, in the national red lists (Boşcaiu & al. 1994; Oltean & al. 1994), the vulnerable status for *Adonis volgensis, Astragalus peterfii, Aquilegia niricans* subsp. *subscaposa, Centaurea reichenbachii, Dianthus spiculifolius* and *Nigritella nigra* subsp. *rubra* and the rare status for *Bulbocodium versicolor, Dianthus petraeus* subsp. *orbelicus* and *Saponaria bellidifolia.*

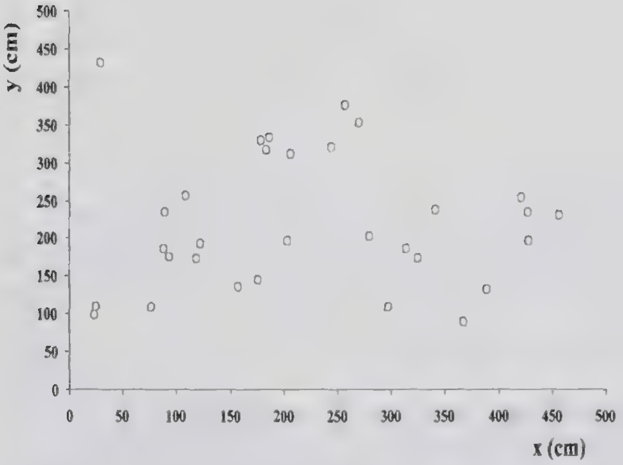

Figure 3. Spatial distribution of Aquilegia nigricans *subsp.* subscaposa *individuals in the grassland (*Agrostio-Festucetum rubrae*) from Tâlva Valley.*

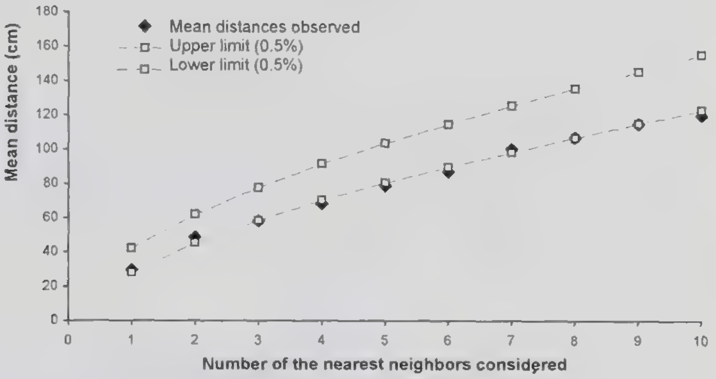

Figure 4. Aggregation tendency of Aquilegia nigricans *subsp.* subscaposa *individuals in the grassland (*Agrostio-Festucetum rubrae*) from Tâlva Valley.*

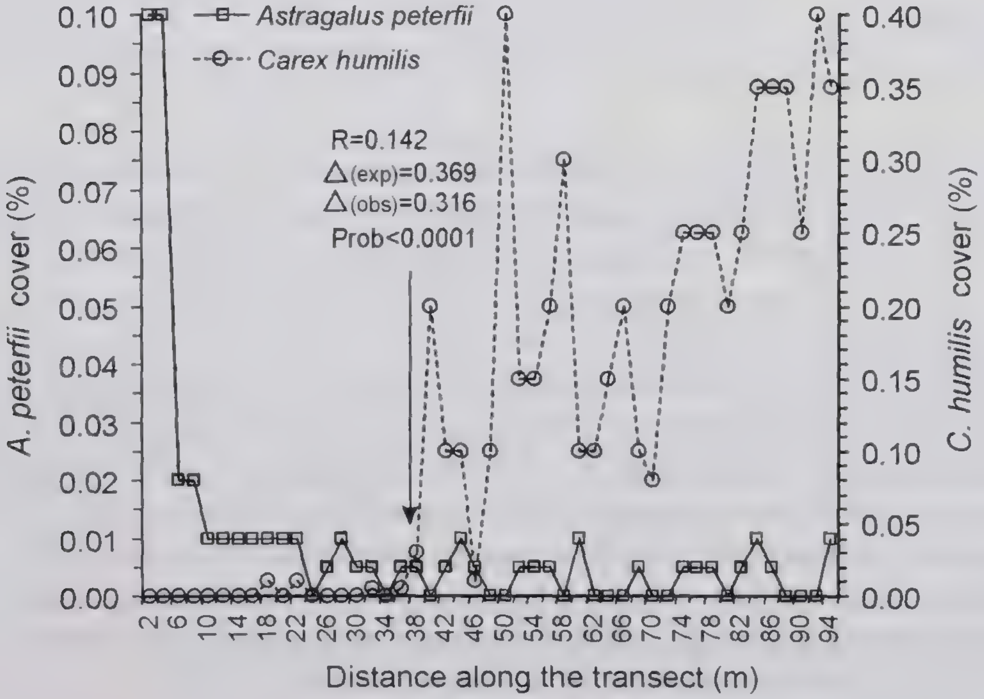

Figure 5. Variation of Astragalus peterfii *and* Carex humilis *cover along the transect.*
The arrow indicates a possible but significant boundary in terms of floristic composition between the two.

Seed germination studies. The observations on the germination of old and fresh seeds from the analyzed experimental groups showed the positive or negative influence exerted by various exo- or endogenous factors. No complete germination studies could be performed for all taxa due to the lack of old seeds and the reduced number of fresh seeds, these being extensively used for *in vitro* multiplications. It was found that depending on the species, age of seeds, storage conditions and treatments applied prior to germination, the germination capacity of the seeds under laboratory conditions was stimulated by constantly applied low temperatures and the number of exposure hours. For the fresh seed of the two taget taxa (*Dianthus petraeus* subsp. *orbelicus* and *Centaurea reichenbachii*), the higest values: 44%, 20%, respectively were recorded after 48 h of exposure. The lowest ones: 5%, 12%, respectively were recorded after 24 h of exposure (tab. 1). The experiments clarly reveal a very low germination capacity on old seeds. The higest values were obtained after 110 h of exposure, both at constantly applied (18%) and low temperature alternatively applied (18% and 16%) (tab. 1).

More significant results were obtained in the case of seeds germinated under classical conditions (in soil, in pots). For the mentioned taxa, the germination capacity values were 78.3% and 75.6% (tab. 1).

Table 1. Germination capacity of *Dianthus petraeus* subsp. *orbelicus* and *Centaurea reichenbachii* seeds under laboratory and classical conditions.
AG, age of the seeds; CLT, constant low temperature; ALT, alternative temperature; SGS, seeds germinated in soil.

Taxon	AG	Germination capacity (%)								
		CLT No. of exposure hours				ALT (12/12h) No. of exposure hours				SGS No. of days
		24	48	72	110	24	48	72	110	150-180
Dianthus petraeus subsp. *orbelicus*	**Fresh**	36	44	40	40	–	–	–	–	78.3
	10 years	4	5	10	18	4	4	4	16	
Centaurea reichenbachii	**Fresh**	12	20	20	20	–	–	–	–	75.6

In addition to the role played by a certain treatment technology, the experiments also showed the importance of the population gene fund, significant differences also appear between the germination capacities of the different populations belonging to the same taxon (e.g. *Centaurea reichenbachii*) (tab. 2).

***In vitro* multiplication.** In the case of *in vitro* multiplication experiments, significant results were revealed for cultures obtained from plantlets resulting from *in vitro* seed germination. The multiplication rates reported being: 2-3 (*Astragalus peterfii, Saponaria bellidifolia*), 12 (*Dianthus petraeus* subsp. *orbelicus*), 110 new plantlets/inoculum, respectively (*Dianthus spiculifolius*). The *Astragalus peterfii, Saponaria bellidifolia* vitro-plants obtained were acclimated in the Botanical Garden.

In vitro cultures posed and still pose problems related to the technique that should be applied to *Aquilegia nigricans* subsp. *subscaposa* and *Centaurea reichenbachii*, regarding in particular the sterilization of explants, which requires the further testing of other methods.

Acclimation of taxa. The living material, collected in the field in the period July-October 2001, was transplanred for the *ex situ* behavior study of the target species. It was found that all individuals flowered

Population	**Germination capacity (%)**	
Exposure (hours)	**Cheile Poşăgii**	**Cheile Runcului**
24 h	12	12
48 h	20	48
72 h	20	16
110 h	20	16

Table 2. Germination capacity of the seeds from two populations of *Centaurea reichenbachii*, under laboratory conditions.

and fruited as early as the first year after their transplantation to hotbeds or to the sectors of the Botanical Garden.

Also, the seeds collected were sown in order to be subsequently multiplied and planted. In the case of *Aquilegia nigricans* subsp. *subscaposa* and *Centaurea reichenbachii*, in classical condition, the recorded germination capacity of the seeds was 75.6%, 83.1%, respectively. (tab. 3). The behavior of plants obtained from seeds and cultivated under different conditions, were observed *ex situ*, for two years (tab. 3).

The viability percentages obtained were different, depending on the species and culture conditions (tab. 3).

After the two years of observation, the highest viability percentage for *Aquilegia nigricans* subsp. *subscaposa* (a perennial taxon, weakly acid-neutrophilic, vegetating on pastures), was found in pots (manure), in the nursery (80% in the first year and 50%, in the second one), while the lowest values were found on the calcareous rocks from the sector of Endemic, Rare and Endangered plants from Romania (45%, 25%, respectively, tab. 3).

In case of *Centaurea reichenbachii* (a biannual, neutro-basophilic species, vegetating on calcareous rocks), the highest values was found on the calcareous rocks (83.3% in the first year and 40% in the second one), while the lowest percentage (36.6; 13.33%, respectively), was found in the bed (garden soil) intended for *Asteraceae* family, from the Systematic sector (tab. 3).

In the first year after planting, the individuals of the two species from all groups formed basal leaves. In the second year the individuals flowered and fructified, except for those cultivated in the Systematic sector, in beds.

The data obtained confirm the ecological preferences of the analyzed species, indicating adequate growth and development only when conditions similar to natural ones are ensured.

Setting-up of the sector of Endemic, Rare and Endangered plants from Romania

As part of a research project (carried out in the period 2000-2002), the sector of Endemic, Rare and Endangered plants from Romania was created. A list of threatened and/or endemic species included in the national and international red lists and conventions,
cultivated in 'Alexandru Borza' Botanical Garden, is available upon request with the author for correspondence of this paper.

This sector was organized to gather species characteristic of various biotopes (pastures, rocks, peat bogs). For this purpose, two rock areas (with calcareous rocks, crystalline schists, respectively), two (oligotrophic and eutrophic) marshes, and an area intended for species characteristic of pastures were created.

The sector gathers, in its special collections, approximately 60 taxa, part of which are cultivated in the other sectors of the garden). Its collections shelter one (2%) extict species (*Osmunda regalis*), one (2%) critically endangered (*Aubrieta intermedia*), three (5%) endangered (e.g. *Aegilops triuncinalis,*

Table 3. Viability of *Centaurea reichenbachii* and *Aqulegia nigricans* subsp. *subscaposa* plants obtained from seeds, under various culture conditions.
P, pots (manure); B, beds (garden soil); CR, calcareous rock.

Taxon	No. of the planted individuals			No. of individuals after one year			No. of individuals after two years		
Conditions of cultivation	**P**	**B**	**CR**	**P**	**B**	**CR**	**P**	**B**	**CR**
Centaurea reichenbachii	30	30	30	22	11	25	10	4	12
Viabilility (%)	-	-	-	**73.3**	**36.6**	**83.3**	**33.3**	**13.3**	**40**
Aquilegia nigricans subsp. *subscaposa*	20	20	20	16	13	9	10	9	5
Viability (%)	-	-	-	**80**	**65**	**45**	**50**	**45**	**25**

Ligularia sibirica, Tofieldia calyculata), 20 (33%) vulnerable (e.g. *Arnica montana, Globularia punctata, Lycopodium clavatum, Muscari commutatum*) and 35 (58%) rare taxa (e.g. *Allium atroviolaceum, Anthericum liliago, Euphorbia myrsinites, Linaria alpina, Nigritella nigra* subsp. *rubra*). Fourteen of all taxa are endemic in Romanian flora (e.g. *Dianthus giganteus* subsp. *banaticus, D. glacialis* subsp. *gelidus, Silene nutans* subsp. *dubia, Viola jooi*).

From all these species twelve are included in the international red lists and conventions: one (*Astragalus roemeri*) is mentioned in IUCN Red List of Threatened Plants (Walter & Gillett 1998) and in Bern Convention (Council of European Communities, 1998); six (e.g. *Aquilegia nigricans* subsp. *subscaposa, Astragalus roemeri, Dianthus petraeus* subsp. *orbelicus, Viola jooi*) in the Globally threatened plants in Europe (World Conservation Monitoring Centre 1998); tree (*Arnica montana, Ligularia sibirica, Lycopodium clavatum*) in Habitats Directive (Council of European Communities 1992) and one (*Ligularia sibirica*) in CITES List (Inskipp & Gillett 2003).

“Alexandru Borza” Botanical Garden encloses in its collections approximately 160 taxa (10.9%) of all 1466 Romanian threatened taxa included in the national and international red lists. In the special threatened plant collection and also in the general ones are preserved three (0.9%) (*Astragalus peterfii A. roemeri, Draba haynaldii*) of the 32 Romanian taxa listed in the IUCN-Red List of Threatened Plants; 16 (16.7%) of the 99 mentioned in the Globally threatened plants in Europe 11 (20%); 11 of the 55 included in Bern Convention; 10 (41.7%) of the 24 mentioned in Habitats Directive; five (13.8%) of the 36 included in CITES List and the single one species mentioned in The World List of Threatened Trees (Oldfield & al. 1998).

Of all preserve threatened taxa one (1%) is listed as extinction (*Osmunda regalis*), three 92%) as critically endangered (*Aubrieta intermedia, Elymus farctus* subsp. *bessarabicus, Schivereckia podolica*), 12 (8%) as endangered (e.g. *Abies alba, Crocus banaticus, Paeonia officinalis* subsp. *banatica*), 57 (36%) as a vulnerable and 87 (53%) are listed as a rare species (e.g. *Cerastium transsilvanicum, Dianthus spiculifolius, Hepatica transsilvanica, Salvia transsylvanica, Trapa natans*). Also, 23 of these threatened species are endemic in Romanian flora (e.g. *Campanula carpatica, Dianthus callizonus, Pinus nigra* subsp. *banatica, Sorbus dacica, Thymus comosus*).

Database

The results obtained will allow the elaboration of a database that will be used for both the editing of the red lists and books and for other programmes of evaluation, monitoring and conservation of phytodiversity. It will have to be compatible with similar databases existing in national and European botanical gardens or research institutes.

It can be concluded that the importance of the existence of threatened plant collections in botanical gardens consists not only in the fact that they conserve a valuable gene fund, which offers the possibility to perform further research, but also in the fact that scientific activity can acquire an educational role, in which case the factors involved in these fields should establish increasingly close connections.

The identification of the adaptive strategies of the endangered species making the object of the present studies could be a basis for the elaboration of plans for the management of protected areas as well as for future *in situ* conservation measures.

We consider that an efficient collaboration among the factors involved in the complex activity of conservation of phytodiversity can also be achieved by the creation of national and European networks specialized in the study of biology and methods for the conservation of threatened species.

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A dry meadow as a live seed bank and an object of research

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Abstract. – *Dry meadows, particularly those in lowlands, are becoming quite rare. In 2002, the Botanical Garden of Ljubljana took a dry meadow on lease. The meadow, covering a surface of 2 ha, lies in the close vicinity of Ljubljana. It has not, for the last 40 years, been subjected to any intensive treatment. The very first sample inventorying of plant species in the course of 2001 revealed the presence of a large number of species; of these quite a number appear in the Slovenian Red Data List of Pteridophytes and Spermatophytes. In the course of the regular monitoring in 2002 the number of determined species increased to 120. The meadow is quite rich in wild orchids, which makes it all the more valuable. It is in the initial stage of overgrowing. A late one-time mowing once a year is expected to contribute to its conservation. The goal of taking the garden on lease and exposing it to proper management is to contribute to an active conservation of this type of habitats and their biodiversity.*

Key words: *active protection, dry meadow, mowing, biodiversity, live seed bank.*

1 Introduction

Dry meadowlands, particularly those in lowlands, are becoming quite rare both in Slovenia and in the world at large. Disappearing along with them is also the diversity of plant and animal species. Changes are evident from associations and species composition, while some of the species are disappearing. These changes result from agricultural intensification – manuring, mowing or abandonment of mowing. Orchids and other geophytes as well as other families (Kaligarič 1977) are particularly sensitive to the introduction of minerals (manuring). In terms of species the degradation of meadows is under a substantial impact of the new technology of fodder preparation, particularly the transition to silage which dictates earlier mowing when meadow plants are not seeding as yet (Šilc 2002).

In 2002, the Botanical Garden of Ljubljana took a dry meadow on lease. The meadow lies at Vižmarje, in the close vicinity of Ljubljana. The peasants had noticed certain plants which otherwise grew at higher areas. The first sample inventory of plant species in the course of 2001 and the fact that in the close vicinity of Ljubljana certain rare species were present in large numbers prompted our effort to preserve this meadow in an as original form as possible. As we learned from its owners, the meadow has not been intensively exploited (neither manured nor mown more than once a year) for the last forty years, which of course heightened our interest in the area. We also learned that the owners of the nearby land were interested in ploughing it up to

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cultivate various field crops. It was therefore imperative to act without delay if all that richness of plants was to be preserved. A single intervention in terms of intensifying the exploitation of the area could destroy the vegetation.

2 Methods

Floristic inventorying was complemented by observation of abiotic factors (dryness in summer time, depth of soil). The reference of the inventoried area according to the Central European mapping is 9852/4 and according to the UTM square VM60. In the course of 2002 the meadow was visited eight times. The plants were determined either in the field or later on in the Botanical Garden. Some of them were transferred to the cultivation area of the Botanical Garden. The rarer species were numerically evaluated, which should enable us to compare the state then with that in the years to come when the meadow will again be mown once a year.

3 Results and discussion

The very first sample inventorying of plant species in the course of 2001 showed the presence of certain species that are not very frequent in the environments of Ljubljana. In 2002 the meadow was under constant monitoring in order to register the current state which will be compared later to the state after a one-time mowing. Quite a number of species also appear in the Red List of Pteridophytes and Spermatophytes (Wraber & Skoberne 1989). All of them fall into the category of vulnerable species highly susceptible to human interventions in the biotope – e.g. *Anacamptis pyramidalis* (L.) L.C.Rich., *Epipactis palustris* (L.) Crantz, *Gladiolus illyricus* Koch, *Gymnadenia conopsea* (L.) R.Br., *Lilium bulbiferum* L. subsp. *bulbiferum*, *Ophrys holosericea* (Burm.fil.) Greuter, *Orchis coriophora* L., *Orchis militaris* L., *Orchis morio* L., *Orchis tridentata* Scop., *Orchis ustulata* L.

According to the current inventory the meadow offers home to more than 120 different species. Our future work will include inventorying and determination of grasses and sedges most of which have not been inventoried as yet.

The area under study can be evaluated through different classifications. According to the palearctic classification of habitat types (Devillers & Devillers-Terschuren 1996) this is a semi-dry Central European meadow rich in orchids (34.3221) which, being a priority habitat, falls under the habitat directive (Council of Europe 1992) so that it has to be considered also in the Natura 2000.

Syntaxonomically, meadowlands of this type are placed into the class *Festuco-Brometea*, order *Brometalia erecti*, and association *Bromion erecti*. The floristic inventories as completed do date point to a more massive presence of the species that count as leading within the association *Onobrychido viciifoliae-Brometum*; a closer determination of the association, however, will also require suitable phytocenological inventories.

Tomažič (1949) described small glade-type pine groves growing on the rough gravel in Posavje, as well as nearby rocky, gravelly meadows mown only once a year, in other words, he also dealed with surfaces not overgrown with pine trees or else the latter grow merely along the margins. He repeatedly refered to a locality known as "Dovjež ob Savi"; judging from the enumerated plant species it would seem fairly certain that he described the very meadow which is the object of our research.

The species considered characteristic of the association (Tomažič 1941) are given in table 1.

The 2002 inventories do not include all of the species cited by Tomažič. The inventorying has not been completed as yet, and it should also be taken into account that the present species composition may have undergone changes so that some species observed in the past may now actually be missing. Noticed among the cited species were *Carex humilis*, *Koeleria pyramidata*, *Ophrys holosericea, Hypochoeris maculata, Trifolium montanum, Linum viscosum* and *Bromus erectus.*

The meadow is in the initial stage of overgrowing. A late one-time mowing wants to contribute to its conservation. It is to be expected that the mowing will be followed by an increased presence of at least some of the species, Orchidaceae in particular. This method of meadow conservation and prevention of overgrowing is resorted to also elsewhere in the world (Fitzgerald 2000).

Table 1. Charcteristic species of the association *Festuceto pseudovinae-Centaureetum rhenanae* Tomažič 1941.
(syn.: Asoc. *Carex humilis-Centaurea rhenana* Tomažič 1940)

Tomažič 1941	**Current name**
C. nitida Host	*Carex liparocarpos* Gaud.
Festuca (*vallesiaca* Schleich. ssp.) *pseudovina* (Asch. et Graebn. Hack. var. *parviflora* Asch. et Graebn.	*Festuca pseudovina* Hack. ex Wiesbaur
Centaurea rhenana Boreau	*Centaurea rhenana* Boreau
Onobrychis viciifolia Scop. subsp. *arenaria* (Kit. em. Koch) Thell. var. *typica* Beck [= *O. arenaria* (Kit.)]	*Onobrychis arenaria* (Kit.) Ser.
Andropogon ischaemum L.	*Andropogon ischaemum* L.
Festuca (*vallesiaca* Schleich ssp.) *sulcata* (Hack.) Nym., Asch. et Graebn. F. *brevifolia* Belli	*Festuca rupicola* Heuff.
Seseli annuum L.	*Seseli annuum* L.
Carex humilis Leyss.	*Carex humilis* Leyss.
Carex caryophyllea Latour	*Carex caryophyllea* Latour
Koeleria (*pyramidata* (Lam.) Domin. ssp.) *montana* (Hausm.) D.T.	*Koeleria pyramidata* (Lam.) PB.
Ophrys fuciflora (Cr.) Rchb.	*Ophrys holosericea* (Burm.fil.) Greuter
Ophrys aranifera Huds.	*Ophrys sphegodes* Mill.
Ophrys muscifera Huds.	*Ophrys insectifera* Huds.
Brachypodium pinnatum (L.) Beauv.	*Brachypodium pinnatum* (L.) Beauv.
Hypochoeris maculata L.	*Hypochoeris maculata* L
Trifolium montanum L.	*Trifolium montanum* L
Linum viscosum L.	*Linum viscosum* L.
Bromus erectus Huds.	*Bromus erectus* Huds.

If the neighbouring parcels of land were no longer exposed to intensive exploitation, they could serve as a source of natural distribution for such species that used to be more common in these parts – as an original re-introduction locality. While inventorying the plants we also collected the seeds of the species for our seed banks. This natural habitat represents a natural reserve of plants which may spread to the neighbouring meadows some of which are intensively exploited for growing different cultures while others serve as intensive producing meadows. As the studied meadow is unique in this area, it is all the more important to take good care of it. It is very close to the actual town area, so it is within easy reach for collecting of seeds and monitoring. It is large enough (2 ha) to represent a natural resource of dry meadow plant species. It serves as a source of seeds for our seed bank as well as a source of live plants in a natural habitat which is used exclusively for observation and research. Mowing it once a year in the middle of August is enough to allow the seeds to ripen and to ensure the reproduction of plants. As seeds are collected in a reasonable quantity, this involves no threat to the natural populations of the species. Weekly monitoring allows us to conserve those among them whose presence in the meadow is less pronounced than that of other species.

4 Conclusions

A long-term objective of the project is to maintain the meadow in the current state, purchase it off or at least arrange for a longer lease contract, and most of all, have it legally protected. This would be extremely useful in view of the fact that the meadow is located within a water collecting area from where a part of Ljubljana is provided with water. The Botanical Garden would there preserve not only the plants in their natural habitat but also the gene resources of the aforementioned population of plants which would serve for research projects and as a source for reintroduction and repopulation of similar impoverished and degraded surfaces but also as a place of live seed bank. The goal of taking the garden on lease and exposing it to proper management is to contribute to an active conservation of this type of surfaces and thus to the conservation of their biodiversity (UNEP 1994).

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The soil seed bank as a resource for *in situ* and *ex situ* conservation of extinct species

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Abstract. *– The importance of soil seed banks and their dynamics for species conservation is demonstrated by using examples from semiaquatic habitats. The differences between the distribution of endangered plants in the underground vegetation (soil seed bank) and actual vegetation cover are shown and discussed in the light of information from "Red data books". Ephemeral species like* Coleanthus subtilis *and* Elatine hexandra *have not been reported for decades in the vegetation cover. Herbarium material encouraged the idea to compare old herbarium material of today highly endangered or extinct species with seeds from soil samples collected in similar localities.*

Key words: *soil seed bank;* Coleanthus subtilis*;* Elatine hexandra*; extinct species;* in situ and ex situ *conservation; biodiversity.*

Introduction

Red Data books document extinction and endangering of species. Most of those species, like the ones growing along the shoreline, need unique conditions to survive. Shoreline plants are able to colonize semiaquatic environments, i.e. conditions alternating between terrestric and hydric (Bernhardt & Poschlod 1993). One characteristic of such plants is their capacity to survive in the soil seed bank during unsuitable conditions. Due to the longevity of seeds, those species remain present for decades (Leck 1989; Bernhardt & Poschlod 1993). Therefore, the soil seed bank contains the species diversity as well as the genetic diversity of populations (Levin 1990; Koch & al. 2003) i.e., serves as "gene bank".

We started by assuming that plant species listed as "extinct" in different regions would still be present in the soil in their original habitat. Under this assumption we analysed soil seed banks of semiaquatic habitats in Northern Germany and Northeastern Austria to see if extinct species could be found.

Areas of study

The study areas were located in Northern Germany and North-eastern Austria (fig. 1) and consisted of the shorelines of fishponds and a number of human-influenced waters. Table 1 shows the location of the sites investigated.

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Table 1. Locations of soil samples.

Location	Region	Character of habtitat	Substrate	Size $(m^2\ ha^{-1})$	N° of samples
Geeste	N. Germany (Lower Saxony)	Gravel pit	Sand	55	8
Biene	N. Germany (Lower Saxony	Gravel pit	Sand	22	3
Laerheide	N. Germany (Lower Saxony	Gravel pit	Sand	28	3
Schönauer Teich (Zwettl)	NE Austria (Lower Austria)	Fishpond	Loamy mud	16	5
Straßendeich (Ritzmannsdorf)	NE Austria (Lower Austria)	Fishpond	Loamy mud	2	5
Hofteich (Ritzmannsdorf)	NE Austria (Lower Austria)	Fishpond	Loamy mud	2	5
Friedenteich (Teichschönau)	NE Austria (Lower Austria)	Fishpond	Loamy mud	3	5

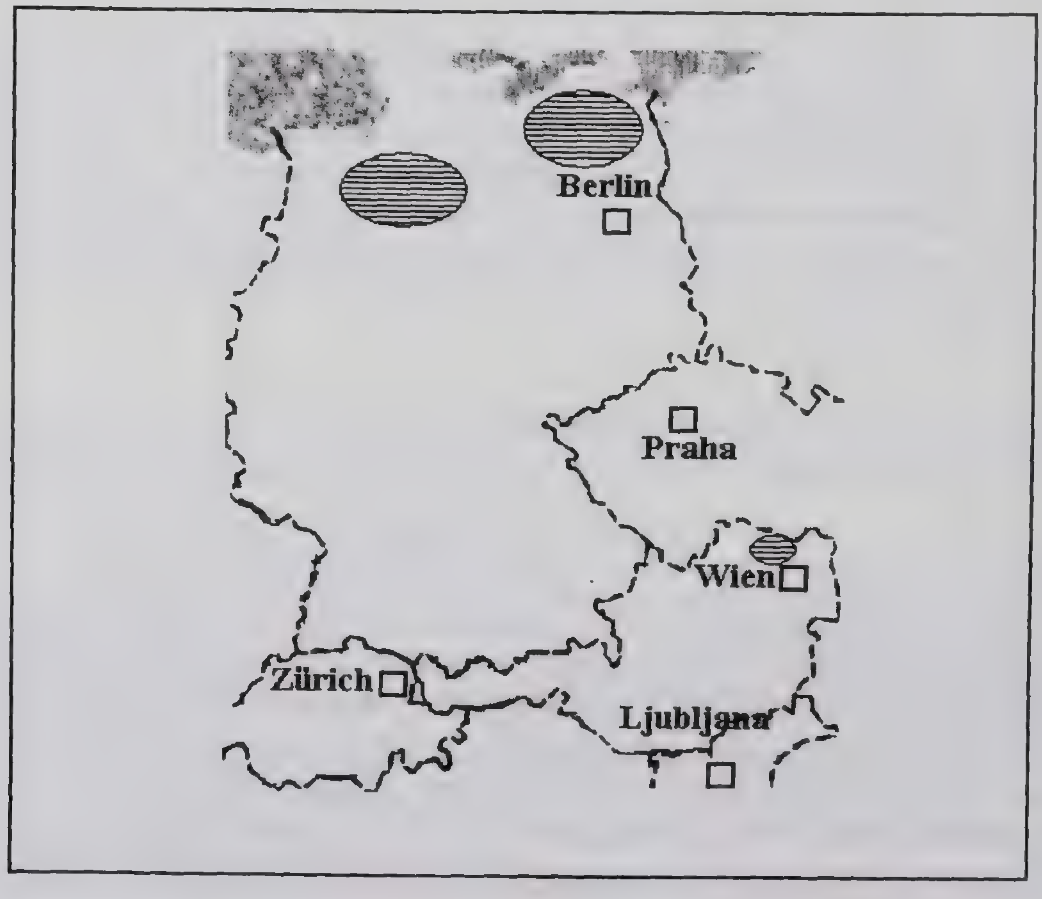

Figure 1. Areas (□) where soil samples were collected.

Target species

Table 2 presents the investigated species and their category of endangering. All *Elatine* species are listed as being extinct in Northern Germany, *Coleanthus subtilis* is listed as being extinct in Austria. All other species are threatened or strongly endangered.

Table 2. Category of endangering (extinction) of the investigated species.
(Niklfeld & Schratt-Ehrendorfer 1999; Garve 1987)

- not distributed 0 extinct or missing 1 threatened 2 strongly endangered 3 extinct

	Category of endangering	
Species	Northern Germany	Austria
Carex bohemica	-	3
Coleanthus subtilis	-	0
Eleocharis acicularis	2	2
Eleocharis ovata	0	2
Cyperus fuscus	1	3
Elatine hexandra	0	1
Elatine triandra	0	1
Elatine hydropiper	0	1
Limosella aquatica	1	2
Scirpus radicans	-	2

Methods

To find habitats of the targeted species we used information from old records and information from herbarium material going back to 1883 from the herbarium of the University of Natural Resources and Applied Life Sciences, Vienna (Scharfetter & Bernhardt 2002); for potential habitats we used topographic maps.

It is an axiom that in sampling populations the lower limit of sample size depends on the size of the organisms being studied and on their sensitivity to damage. Since buried seeds can be considered to be almost infinitely tiny, the sample size for seed bank studies should theoretically be very small. However, if individual samples are to be processed separately, which is timeconsuming, it is not practical to use very small sample sizes. On the other hand, relatively few large samples often produce unacceptably large sample variances. The two possible ways out of this dilemma are to make the samples as small as possible, or take very small samples and pool them (e.g. Thompson & al. 1996).

Throughout the area of occurrence of the vegetation type, randomised plots of 1 m^2 each were established, each representing one sample. From each plot, 25 samples were taken at random to a depth of 20 cm with an auger of 2.6 cm diameter; this was repeated a second time and soil samples were subsequently bulked by plot (for detailed discussion of sample sizes, probe volume and statistics see Thompson & al. 1996 and references therein).

The bulked soil samples were analysed by two methods: the first consisted of separating the organic fraction by washing and floating and then recovery by sieving, followed by counting the seeds under a microscope. For the second method, the soil samples were spread out in the greenhouse and germinated seeds counted and the seedlings identified. The results were combined in pairs and summarized (Bernhardt & Hurka 1989).

Results

We found that a number of individuals are represented in the soil seed bank although species like *Coleanthus subtilis* or *Elatine* spp. are listed as extinct. These species are still living *in situ* with a high density of seeds in the soil seed bank. The results of the soil seed bank investigations are summarised in table 3.

Firstly, seeds of ephemeral species are present in the soil seed bank in high numbers without appearing in the vegetation cover. *Coleanthus subtilis* and *Elatin*e spp. are showing the highest densities. Both species produce a high number of very small seeds with the capacity to survive for a long time in the soil building up a reservoir of biodiversity (Bernhardt 1995; Poschlod 1996).

Secondly, there are differences between the recovery and the germination method used to extract the seeds from the soil. Own experience (Bernhardt, in preparation) shows that for ephemeral species higher densities by spoiling and sieving are found. By using that method more semiaquatic species were found in the soil in Northeastern Austria than in Northern Germany (Lower Saxony).

Table 3. Average number of seeds from soil samples (estimated per m^2) by comparing two methods of extraction.
* species combined due to difficulties in determination

Number of sample plots (two repiclates each)	Northern Germany		Austria	
	12	12	5	5
Species	**Spoiling method**	**Germination method**	**Spoiling method**	**Germination method**
Carex bohemica	-	-	97.310	41.820
Coleanthus subtilis	-	-	157.329	16.306
Eleocharis acicularis	248.500	116.712	98.510	36.820
Eleocharis ovata	-	-	65.700	21.970
Cyperus fuscus	120	-	-	-
Elatine hexandra *	93.401	11.863		
Elatine triandra *	22.308	1.012	261.270	31.422
Elatine hydropiper *	-	-		
Limosella aquatica	-	-	24.700	13.310
Scirpus radicans	-	-	12.200	8.060

Such specialized seed populations germinate in exposure experiment over a long period, mostly years. Therefore spoiling and sieving it is better for the inventory than to use the germination method. But time and workload is much higher in the former (cf. Thompson & al. 1996).

Generally, the soils along the shoreline of Austrian fishponds contain more ephemeral pioneer species than in Northern Germany with its lower number of species. The diversity of species is higher in the Midcentral Europe where fishpond management has a longer tradition than in Northern Germany.

As the results demonstrate, these habitat types and its endangered vegetation can still effectively be conserved by traditional fishpond management methods.

Conclusions

Our investigations showed that species listed as extinct or highly endangered can recover in the soil seed bank (Bernhardt 1995, 1999). This *in situ* storage can be used for revegetation in cooperation with management methods. Semiaquatic species, having high reproduction rates as is characteristic for colonizing species, are able to survive for a long time.

The reason why they do not grow up are a lack of water level fluctuations (Hejny 1962, 1969; Bernhardt 1999) caused by the intensification of fish-hatching. For *in situ* conservation it is necessary to biannually drain ponds during winter and refill at the beginning of summer (compare Poschlod 1993, 1996). Our results demonstrate that these habitat types and its endangered vegetation can still be effectively conserved by traditional fishpond management methods.

But anthropogenic waters like those found in gravel pits can be managed to conserve populations of endangered or extinct species *in situ* (Bernhardt 1999).

For *ex situ* conservation seeds extracted from soil samples can be stored as can be dugouts of soil containing seeds. Those samples can be used to re-vegetate new habitats or former sites (Bernhardt 1995).

For conservation collections it seems better to collect and store soil samples parallel to collecting seeds from the mother plants. Seeds for conservation collections can also be recovered using the sieving method. Germination tests should be carried out to establish viability before storage (Bernhardt & Hurka 1989). As regards the methods used for analysing soil seed banks, in light of the comparison between the two methods the sieving method is better.

For conservation purposes monitoring of populations is necessary (Ballou & al. 1995; Bernhardt 1996), including investigations into the problem of lacking gene flow and loss of genetic diversity (Koch & al. 2003).

For conservation practice and *ex situ* conservation it will be necessary to know how long soil samples can be stored without the seeds loosing viability. Those results should make an establishment of a gene bank for ephemeral species, especially for those mostly found in the soil seed bank, possible.

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Evaluation of some morphological characters of *Aegilops cylindrica* populations from Hungary

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Abstract. – *In order to assess the genetic diversity of some jointed goatgrass (*Aegilops cylindrica *Host) populations in Hungary, some of their morphological traits and agronomic characters were evaluated in 2001-2002. Jointed goatgrass is a wild relative of wheat and the only native* Aegilops *species present in the Hungarian flora. A total of 54 samples from natural populations at 19 sites were collected (1998-2002) in the central and northern parts of Hungary. For detailed morphological characterisation, 23 samples were selected and evaluated in an autumn sown field trial in three replications on meadow loam soil. Multivariate statistical methods were used to analyse the association of some morphological traits and agronomic characters (plant height, length of ear, number of spikelets/ear, number of seeds/ear, grain diameter, thousand seed mass). Variation was detected in both morphological and grain characters. The experimental data indicate the existence of considerable genetic variation underlying the traits studied in jointed goatgrass populations grown in the experiments conducted.*

Introduction

The centre of diversity for *Aegilops cylindrica* Host is from the northern coastline of the Eastern Mediterranean to Central Asia. Jointed goatgrass is also found in the Balkan peninsula, Carpathian basin, Crete, Turkey, Caucasia, Iran, Iraq and Afghanistan. It has been reported as an adventive species in several countries of Western and Central Europe (Tutin & Humphries 1980, van Slageren 1994). At the end of the 19th century this species was introduced in the United States of America. *Aegilops cylindrica* (fig. 1) is the only species within the genus *Aegilops* that is native in the Hungarian flora (Soó 1973) and closely related to common wheat (*Triticum aestivum*). It grows in fallow fields and along slopes of hillsides, wastelands and pastures. Sometimes, it occurs on the borders of cultivated *Triticum aestivum* fields and spontaneous hybrids are produced. Degen (1917) first described a hybrid between *Aegilops cylindrica* and *Triticum aestivum* [x *Aegilotriticum sancti-andreae* Soó] in Pest County near the town Szentendre in central Hungary. This natural hybrid was also found in Turkey, Armenia, Turkmenistan, Iran, Uzbekistan and Crimea (van Slageren 1994). The Institute for Agrobotany has conducted regular collecting work in different parts of Hungary for studying genetic diversity of this species since 1994. The main aim of the present study was to analyse the variation for some morphological characters among populations originated from Hungary.

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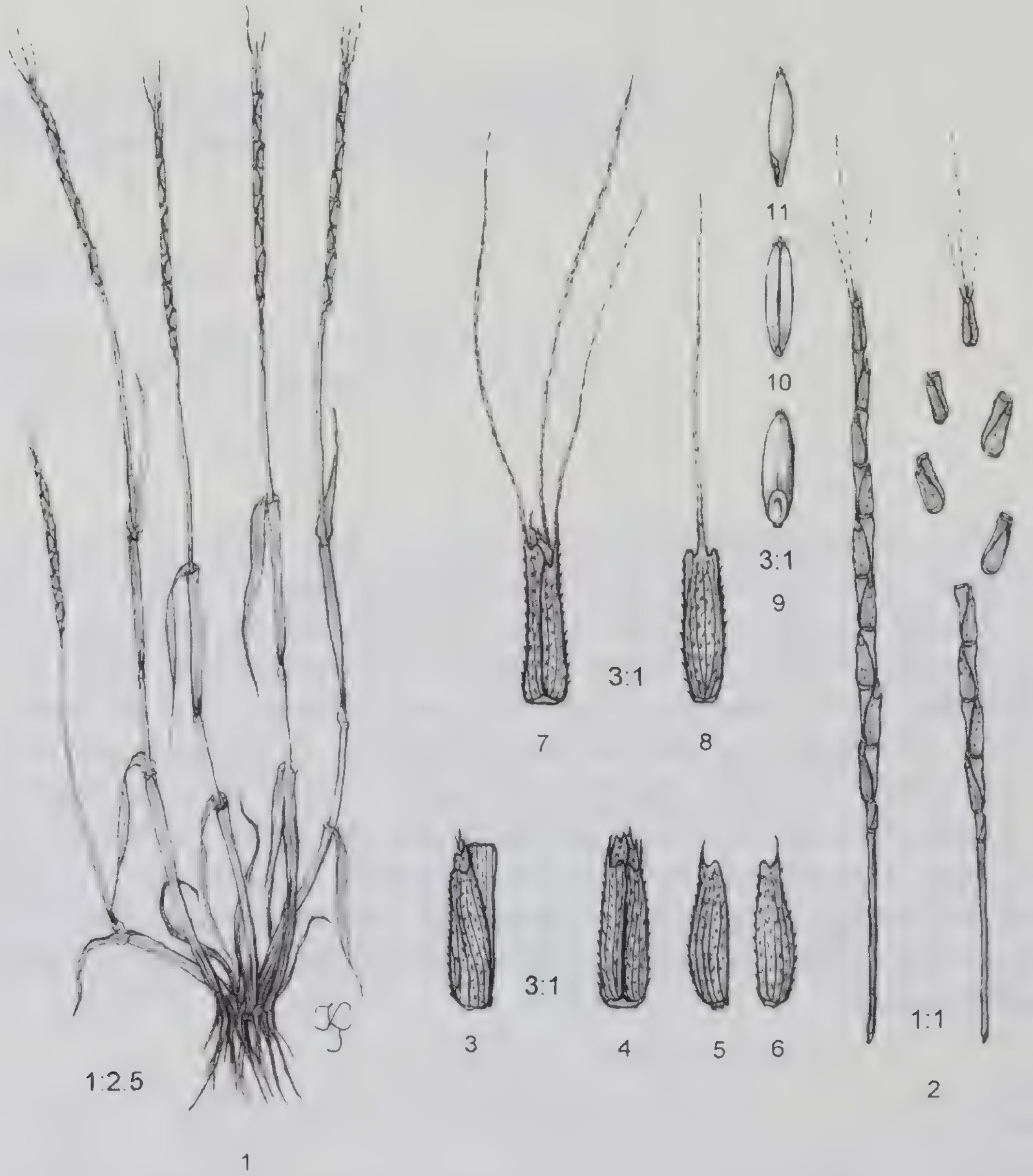

Figure 1. Aegilops cylindrica*: 1. habit; 2. spike; 3-4. spikelet; 5-6. glume; 7. apical spikelet; 8. glume of apical spikelet; 9. dorsal surface of seed; 10. ventral surface of seed; 11. lateral surface of seed; (drawing by T. Kovács from site Bokros 1, 1998).*

Material and methods

Collecting trips were planned to different sites where jointed goatgrass has expected to occur in the middle and northern parts of Hungary (Vörösváry 1998). This area is part of the Great Hungarian Plain and boardering hilly territories of Mátra-mountains and north of Budapest (fig. 2). Collections were undertaken in June and July 1998-2002 and included areas from 46° 25' N to 48° 07' N and from 19° 02' E to 21° 21' E. Populations were collected from low (82-88 m.a.s.l.) to moderate (99-220 m.a.s.l.) altitudes. In order to obtain representative samples and to maintain the original composition of the natural populations, specimens were collected at random.

During this field work, 54 accessions from 19 sites were collected and subsequently stored in the active germplasm collection of the Institute for Agrobotany (table 1).

Grain samples from 23 populations (table 1) collected in natural habitats were planted in field trials in 2001-2002 with three replications for studying morphological variability under the same climatic and ecological conditions. Plants were grown in experimental plots at the Institute for Agrobotany, Tápiószele, located in the north-west part of the Great Hungarian Plain. This location is characterized by a cold winter and a dry and hot summer. Populations evaluated (see table 1) were sown in autumn on rich meadow loam soil. Ten plants were evaluated for nine morphological characters from each plot (growth habit, upper leaf blade length, upper leaf blade width, plant height, length of ear, number of spikelets per ear, number of seeds per ear, grain diameter, thousand seed mass). Statistical analysis was performed using SPSS version 8.0 software. For evaluation of morphological characters IBPGR descriptors list for wheat (IBPGR 1985) were applied.

Table 1. Accessions of *Aegilops cylindrica* collected in Hungary.
Evaluated populations are indicated with an asterisk in column 1.

No.	Accession number	Site of collection	Year of collection	Latitude	Longitude	Altitude	Distribution pattern
* 1	RCAT073122	Bokros 1	1998	46°45'	20°03'	99	abundant
* 2	RCAT073123	Bokros 2	1998	46°45'	20°03'	86	abundant
* 3	RCAT073124	Bokros 3	1998	46°45'	20°03'	84	abundant
* 4	RCAT073125	Bokros 4	1998	46°45'	20°03'	84	isolated
5	RCAT074314	Lakitelek	1998	46°52'	20°00'	86	isolated
6	RCAT074318	Csépa	1999	46°42'	20°05'	84	isolated
* 7	RCAT074329	Mártély	1999	46°25'	20°19'	82	isolated
* 8	RCAT054307	Budapest	2000	46°25'	20°19'	83	abundant
* 9	RCAT053739	Fót 1	2000	47°37'	19°11'	161	abundant
* 10	RCAT053740	Fót 2	2000	47°37'	19°12'	176	abundant
* 11	RCAT053741	Fót 3	2000	47°37'	19°12'	182	isolated
* 12	RCAT053742	Fót 4	2000	47°37'	19°12'	195	isolated
* 13	RCAT054308	Abasár 1	2001	47°47'	19°58'	220	isolated
* 14	RCAT054309	Abasár 2	2001	47°47'	19°58'	220	isolated
* 15	RCAT054318	Bér 1	2001	47°52'	19°29'	194	isolated
* 16	RCAT054303	Bokros 1	2001	46°45'	20°03'	99	isolated
* 17	RCAT054304	Bokros 2	2001	46°45'	20°03'	86	isolated
* 18	RCAT054307	Budapest	2001	47°30'	19°40'	102	isolated
* 19	RCAT054306	Cserebökény	2001	46°46'	20°26'	85	abundant
* 20	RCAT054311	Csörög	2001	47°44'	19°11'	107	abundant
21	RCAT054338	Markaz	2001	47°48'	20°02'	210	isolated
22	RCAT055380	Nagytőke	2001	46°43'	20°14'	84	isolated
* 23	RCAT054305	Öcsöd	2001	46°53'	20°22'	85	isolated
* 24	RCAT054313	Pócsmegyer	2001	47°42'	19°06'	111	isolated
* 25	RCAT054316	Pomáz	2001	47°38'	19°02'	111	isolated
* 26	RCAT054314	Szentendre	2001	47°41'	19°02'	147	isolated
* 27	RCAT054312	Tahitótfalu	2002	47°44'	19°06'	117	isolated
28	RCAT056375	Abasár 1	2002	47°47'	19°58'	220	isolated
29	RCAT056519	Abasár 2	2002	47°47'	19°58'	220	isolated
30	RCAT056376	Bér 1	2002	47°52'	19°29'	194	abundant
31	RCAT056377	Bér 2	2002	47°52'	19°29'	198	abundant
32	RCAT056520	Bokros 1	2002	46°45'	20°03'	99	isolated
33	RCAT056321	Bokros 2	2002	46°45'	20°03'	86	isolated
34	RCAT056378	Bokros 3	2002	46°45'	20°03'	84	isolated
35	RCAT056522	Csépa	2002	46°42'	20°05'	84	isolated
36	RCAT056379	Cserebökény	2002	46°46'	20°26'	85	isolated
37	RCAT056380	Csörög	2002	47°44'	19°11'	134	isolated
38	RCAT056381	Csörög 2	2002	47°44'	19°11'	134	isolated
39	RCAT056382	Csörög 3	2002	47°44'	19°11'	149	isolated
40	RCAT056383	Fót 1/1	2002	47°37'	19°11'	161	isolated
41	RCAT056523	Fót 1/2	2002	47°37'	19°12'	192	isolated
42	RCAT056384	Fót 2/1	2002	47°37'	19°12'	176	isolated
43	RCAT056385	Fót 2/2	2002	47°37'	19°12'	176	isolated
44	RCAT056386	Fót 3	2002	47°37'	19°12'	182	isolated
45	RCAT056524	Fót 4	2002	47°37'	19°12'	195	isolated
46	RCAT056387	Gyöngyöstarján	2002	47°19'	19°58'	174	abundant
47	RCAT056525	Mártély	2002	46°29'	20°12'	88	isolated
48	RCAT056388	Öcsöd	2002	46°53'	20°22'	85	isolated
49	RCAT056389	Pomáz	2002	47°38'	19°02'	111	isolated
50	RCAT056390	Szentendre 2	2002	47°41'	19°02'	147	isolated
51	RCAT056391	Szentendre 3	2002	47°41'	19°02'	184	abundant
52	RCAT056392	Szentendre 4	2002	47°41'	19°02'	183	abundant
53	RCAT056393	Tahitótfalu	2002	47°44'	19°06'	117	isolated
54	RCAT056394	Tokaj	2002	48°07'	21°21'	215	abundant

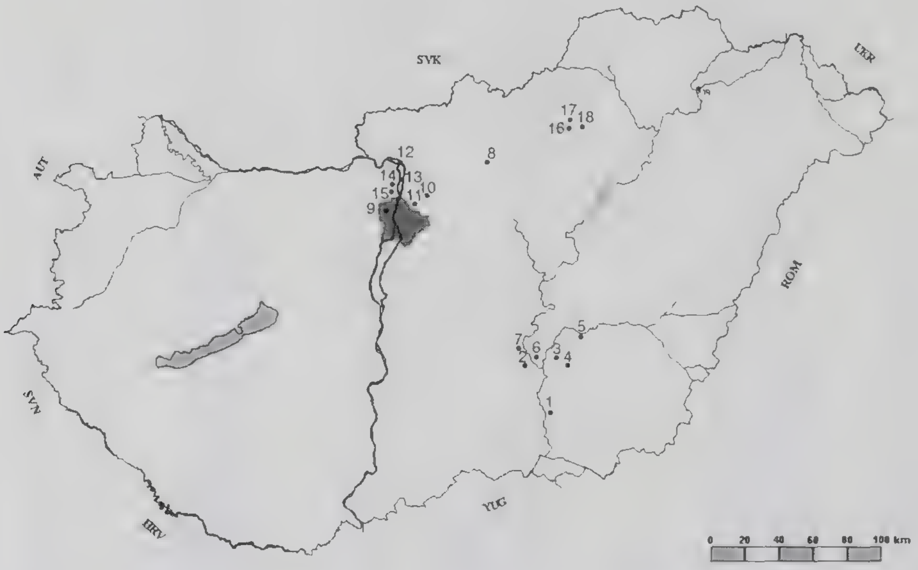

Figure 2. Map of collecting sites for Aegilops cylindrica *in Hungary.*
1. Mártély, 2. Bokros, 3. Nagytõke, 4. Cserebökény, 5. Öcsöd, 6. Csépa, 7. Lakitelek, 8. Bér, 9. Budapest, 10. Csörög, 11. Fót, 12. Tahitótfalu, 13. Pócsmegyer, 14. Szentendre, 15. Pomáz, 16. Abasár, 17. Gyöngyöstarján, 18. Markaz, 19. Tokaj

Results and discussion

In order to characterize differences among *Aegilops cylindrica* populations, distribution patterns during the collecting missions were assessed. The distribution pattern varied from isolated few plants to abundant occurrence (table 1). Twenty-three populations were selected for field trials to evaluate morphological characters. Considerable differences were found for the most traits studied. Analysis of growing habit revealed a high percentage of intermediate and prostrate plants (fig. 3). Table 2 summarizes the parameters for each quantitative variable. The upper leaf blade average length ranged from 6.87 cm (Bokros 3/98) to 8.98 cm (Budapest /01). The upper leaf blade mean width ranged from 0.56 cm (Bokros 3/98) to 0.61 cm (Budapest /01). Average plant height varied considerably between populations ranging from 37.67 cm (Cserebökény /01) to 46.52 cm (Fót 1/01) (fig. 4).

Data for the most important spike characters (length of ear, number of spikelets per ear, number of seeds per ear) indicated high diversity at population level.Length of ear showed marked differences between populations from the same locality (from 3.6 cm to 12.8 cm Abasár 2/01, from 7 cm to 11.3 cm Abasár 1/01 respectively). Length of ear varied from 9.76 cm to 11.32 cm. The grain production characters showed considerable variation among populations. For example, the number of spikelets per ear in population from Abasár 2/01 varied from 4 to 12 spikelets. Average number of spikelets per ear varied from 8.73 (Bér /01) to 10.23 (Budapest /01). Average number of seeds per ear ranged between 12.6 (Fót 4/01) to 17.96 (Budapest /01) grains (fig. 5). Grain diameter ranged from 0.11 cm to 0.30 cm (fig. 6). Thousand seed mass values (from 7.57 g to 11.89 g) indicated a high level of diversity (fig. 7).

The morphological traits contributing to ecological distribution of diversity were leaf length and plant height in *Aegilops cylindrica* populations from central part of Great Hungarian Plain (Bokros) and near of Budapest. Morphological variation between jointed goatgrass populations originated from other parts of Hungary (Transdanubia, Gödöllõ hills) was also observed by Vörösváry & al. (2000). The analysis of variance on morphological traits (leaf width, leaf length, plant height, thousand seed mass) indicating high level of variation was reported from other part of the geographical distribution of *Aegilops cylindrica* (Zaharieva 2003).

Table 2. Statistical parameters for quantitative characteristics.
SD = standard deviation; CV = coefficient of variation.

Character	Range	Mean	SD	CV (%)
Morphological				
Upper leaf blade length (cm)	6.87-8.98	7.98	0.5105	6
Upper leaf blade width (cm)	0.36-0.61	0.46	0.0738	16
Plant height (cm)	37.67-46.52	41.54	2.4106	6
Length of ear (cm)	9.76-11.32	10.3	0.4685	5
Spikelet per ear (no.)	8.73-10.23	9.35	0.3538	4
Seed per ear (no.)	12.63-17.96	15.52	1.3939	9
Grain				
Grain diameter (cm)	0.11-0.3	0.19	0.06	31
Thousand seed mass (g)	7.57-11.89	9.44	1.0743	11

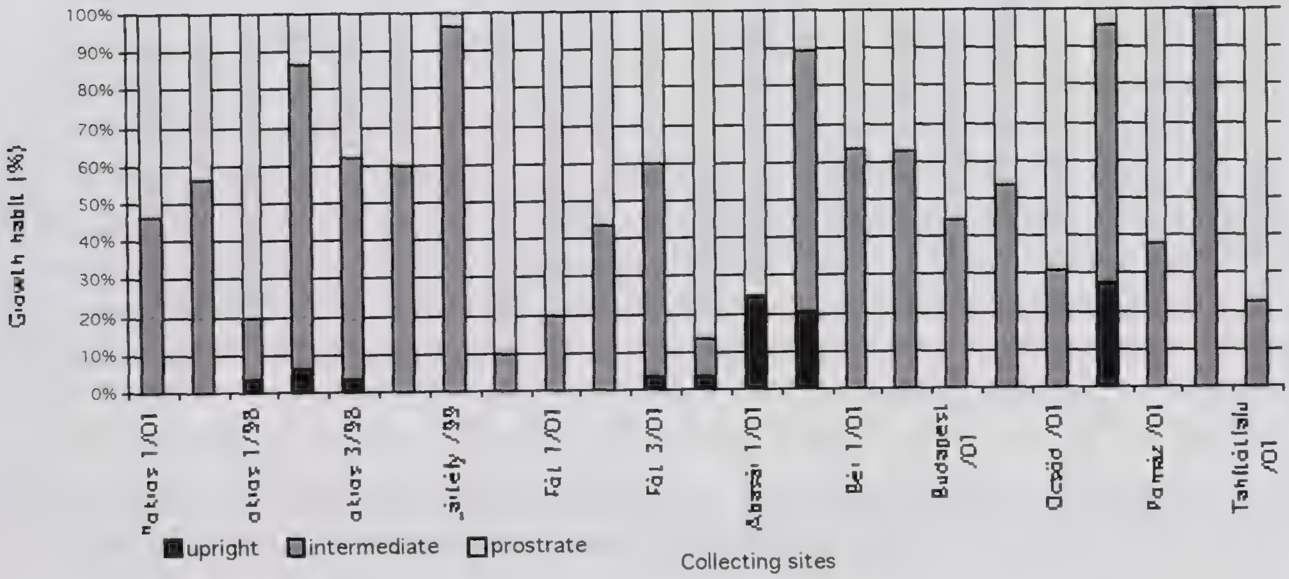

Figure 3. Distribution of growing habit at the evaluated Aegilops cylindrica *populations.*

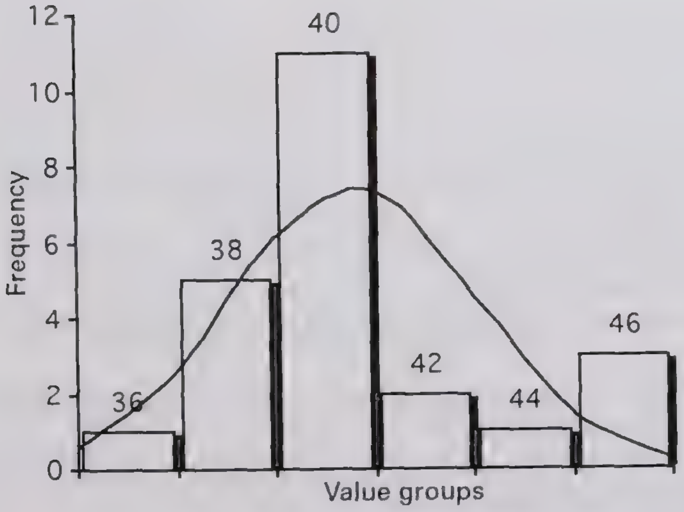

Figure 4. Distribution of plant height in Aegilops cylindrica *populations.*

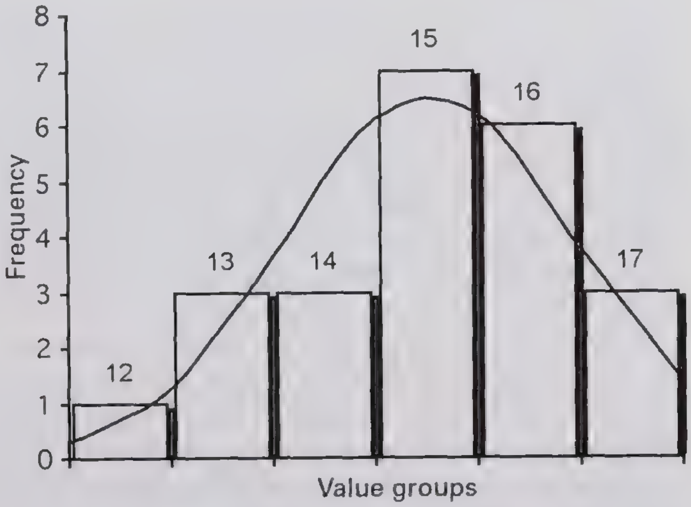

Figure 5. Distribution of number of seeds per ear in Aegilops cylindrica *populations.*

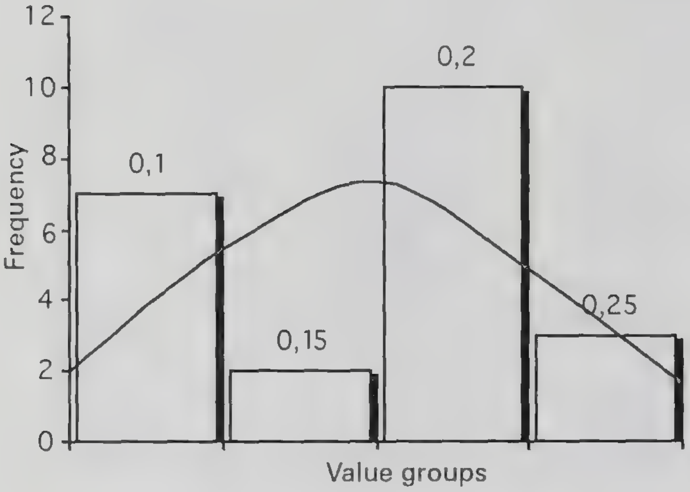

Figure 6. Distribution of grain diameter in Aegilops cylindrica *populations.*

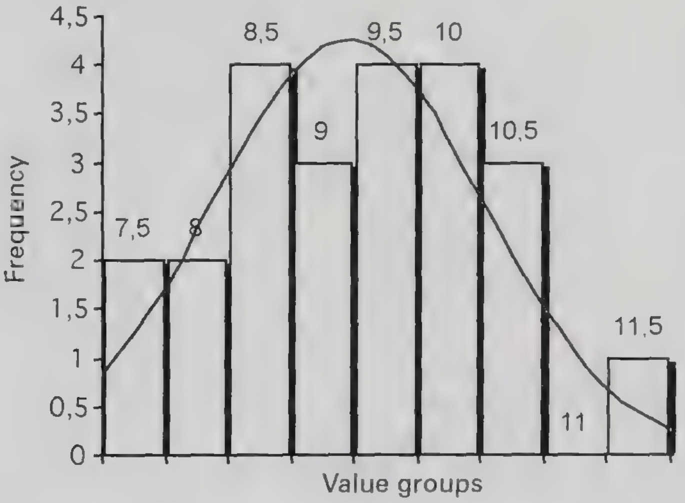

Figure 7. Distribution of thousand seed mass in Aegilops cylindrica *populations.*

Conclusion

Results of this study provide information on the extent of variation and geographic pattern of important morphological traits. Our investigations suggested that in the examined *Aegilops cylindrica* populations upper leaf blade width, number of seed per ear and grains characters (grain diameter, thousand seed mass) contributed more than other traits to the diversity at population level. Data obtained are used to analyse the distribution of genetic diversity and to develop strategies for sampling and *in situ* conservation of this species in Hungary.

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The conservation biology of creeping marshwort (*Apium repens*) in Belgium: aims, methods and first results

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Abstract. *– A research project on the conservation biology of the rare* Apium repens *in Belgium is presented. The species is still present in five localities, whereas it has certainly occurred in at least 24 localities in the past. The (aut)ecology (soil nutrient levels and hydrology) and phytosociology of the species are studied within the Belgian populations. Presence/absence monitoring is carried out as well as demographic monitoring in order to determine the trend in population size, the plant density, and the number of flowers and of seeds produced per plant, and to relate these to soil water level and grazing pressure. Methods are given in some detail. Results show that the surface area of four localities in which the species grows ranges from 5 m*^{2} *to 6 ha. Within these areas A. repens is present with average densities ranging between 1 and 200 ramets per m*^{2}*. Locally, densities up to more than 2200 ramets per m*^{2} *were found in frequently and short cut lawns of a public park. The disturbance effected by the mowing appears to offer optimal conditions, leading to plant densities that equal or surpass those in naturally optimal conditions. In another part of the investigation, uncertain taxa are identified by molecular techniques and the genetic variation within and between the populations is measured.*

Key words: conservation biology, rare plant species, monitoring, population density, ramets

Introduction

Apium repens (Jacq.) Lag. is a plant species from open, moist habitats. Its main distribution lies in northwest Europe and it is also present in adjacent southwest Europe and northwest Africa (Hulten & Fries 1986). It is rare or endangered throughout its area (Vogel & Büscher 1988; Frank 1990; Mierwald 1990; Grassly & al. 1996; Maas 1999). It is mentioned in annex II and annex IV of the Habitat Directive of the European Union (Directive 92/43/EEG), listing the species of community interest in need of strict protection. In Belgium it is a very rare species (Lambinon & al. 1998). Aiming at developing a conservation strategy for this species, we initiated a research programme on its conservation biology. This contribution gives an overview of the different parts of the programme and describes the methodology that is followed and the first results.

Identification of taxa and population genetics

The realisation of recovery plans for a species takes several steps, of which the first is the gathering of basic information about inter alia nomenclature and taxonomy (Banares & al. 1995). *A. repens* is known

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to be often confused with some forms of *Apium nodiflorum,* and intermediary forms occur (Grassly & al. 1996). According to some authors, these forms would be hybrids (Riddelsdell 1917), but most authors consider them as a variety of *A. nodiflorum* (e.g. Fabri 1991). Moreover, *A. repens* is sometimes confused with *A. inundatum*, especially the form *isophyllum* (Sonder) Thell. (Magnel 1925; Reduron & Wattez 1986). In order to characterize these taxa and to identify critical specimens, molecular markers are used, with material from geographically diverse sources.

Knowledge of level and distribution of genetic variation within and between populations is important for designing efficient and sound conservation policies. Therefore, a population genetic study using different molecular markers (cpDNA PCR-RFLP, ISSRs and RAPDs) will be undertaken.

Chorology

A second step for a recovery plan includes the investigation of the past and present distribution of the species, in order to be able to assess its present status and the trend of its degree of rarity. This was done for *A. repens* in Belgium by a thorough search in the floristic literature and in all public Belgian herbaria, and by field work. All localities in Belgium where the species had been observed since 1970 were visited in order to check the presence of the species. It was found that it was still present in only five isolated populations, whereas in the past, there have been reports of it in at least 53 Belgian localities. From these, 24 are certain, since herbarium material was available; these are all located in the northern part of the country. The remaining populations vary much in size, the surface area occupied by the species going from 5 m^2 to 6 ha (Ronse 2004, in press).

(Aut)ecology and phytosociology

Further steps are the investigation of the ecology and phytosociology of the species. More specifically, samples of the top soil from population sites have been taken and analysed (pH, humus, P, K, Mg, Ca and Na). The soil water level is measured every two weeks in the main populations. Phytosociological relevés have been taken and will be compared to similar relevés from abroad. The presence and importance of soil seed banks will also be investigated.

Monitoring of the populations

Four of the five Belgian populations of *A. repens* are monitored. The populations have a different size. The presence/absence of the species is monitored in permanent plots once a year, so that the trend of the population size can be estimated. Moreover, a demographic monitoring scheme has been devised, in order to analyse the status and age structure of the populations, as well as the factors controlling growth and reproduction. This is carried out monthly from April to October, counting individual plants. For both kinds of monitoring, plots are located using two-stage sampling (Elzinga & al. 2001): primary sampling units of 50 m x 10 m are randomly located in the sampled populations. Within these, secondary sampling units (permanent quadrats) are randomly located: four quadrats of 2.25 m ^{2} for the presence/absence monitoring and one quadrat of 0.5 m x 2 m or 0.5 m x 4 m for demographic monitoring. The latter quadrats are marked permanently: two corners are positioned by metal pegs of 1 m sunk into the ground, flush with the soil. As *A. repens* is a small creeping perennial that forms numerous ramets, it is mostly impossible to distinguish between different genets (ramets originating from different plants). Thus, ramets are recorded individually. The number of ramets within the plots is counted monthly, their developmental stage (seedling, vegetative stage, with flowering buds, flowering stage, flowering finished, with seeds) is noted and their position is recorded. The number of seeds per inflorescence is also counted. The position of the ramets is recorded in a 1m x 0.5 m aluminium grid frame (fig. 1). Its design is inspired and adapted from Bradshaw (1981). It has a loose cross-bar with 1 mm intervals inscribed and numbered; both long sides of the frame also have inscriptions of the length measures. In case of very high plant densities, individual positions are visually drawn on a transparent, weatherproof

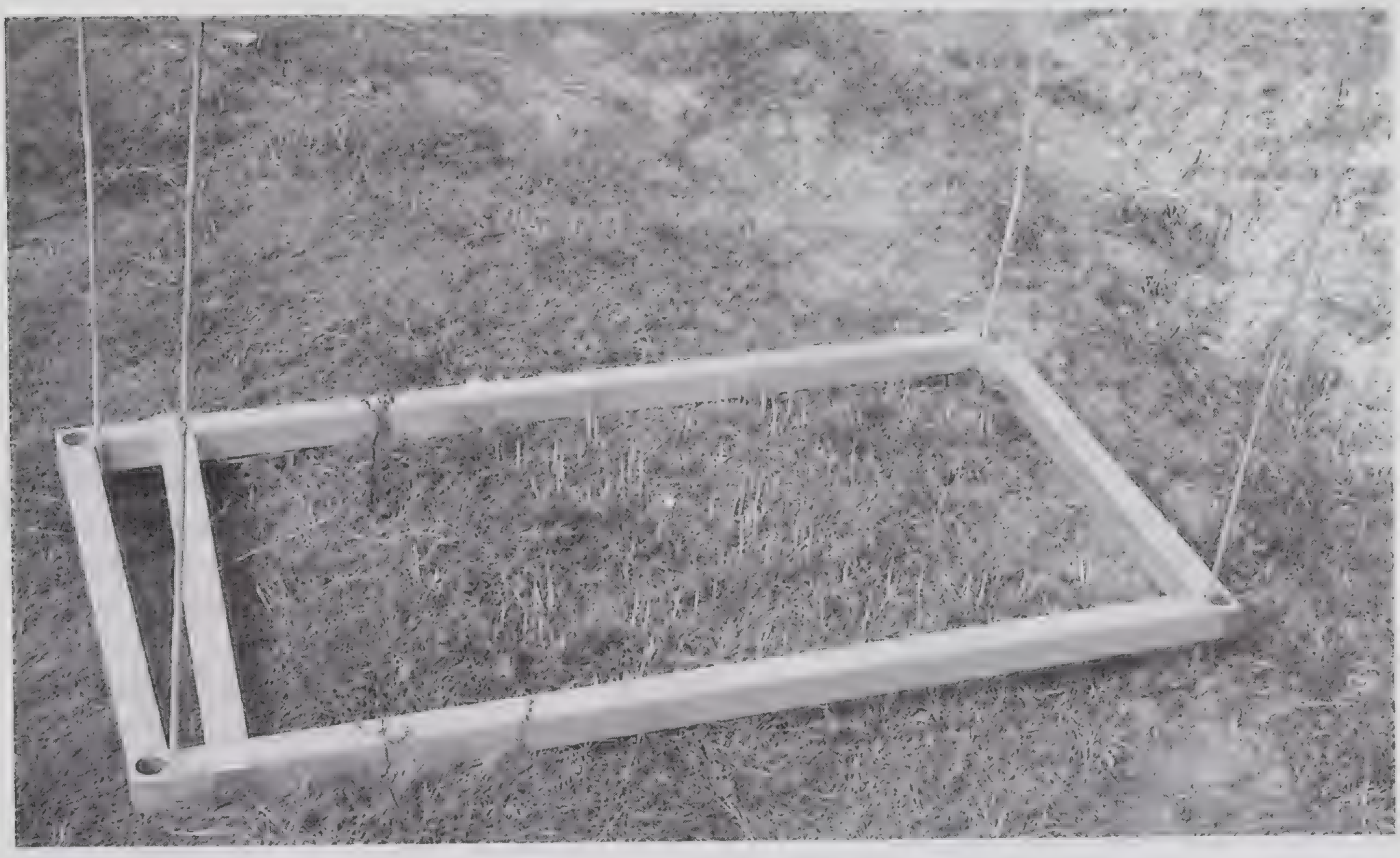

Figure 1. Aluminium grid frame designed to measur the position of individual plants or ramets for demographic monitoring.

plastic film, fixed on the frame with adhesive tape. Special leads (Staedtler Glasochrom) are used that can write on this film even when wet; every month a different colour is used.

It is aimed to relate these data to the soil water levels, where available, and to the grazing pressure or mowing regime.

Some results concerning the size of the population and the plant densities are already available. Plant densities are expressed as numbers of ramets in the surface area occupied by the species, except when the term 'global density' is used, which involves the total area of the land entity where the species is found, eg a meadow. The three smallest populations all are coastal populations that lie in or close to the dunes; they are grazed by cows, horses or donkeys. In the smallest population *Apium repens* occurs within a surface area of 5 m^2, with estimated numbers of between 500 and 1000 ramets. Two other populations cover a surface area of 20 m^2 and resp. 12 000 m^2. The latter is situated in a meadow of 2.8 ha, where the species occurs with a plant density between 10 and 20 ramets per m^2 ; the total number of ramets present in this population must be several ten thousands. The two remaining populations are large and lie in inland marshes. One population lies in common land grazed by cows and horses, that is periodically flooded by a nearby river. *Apium repens* occurs here over an area of about 3 ha, within a meadow of 16 ha; the density of the ramets mostly lies between 2 and 4 ramets per m^2, with locally numbers up to 380 ramets per m^2. The global density for the meadow is estimated on 6000 ramets per ha (0.6 per m^2) and the total number of ramets in this population is estimated to approximate one hundred thousand. The largest population occurs in an area that had traditionally been used as common land, but that has been a public park since 1930. *A. repens* occurs in most of the lawns, over an area of about 6 ha. The average plant densities for the lawn lie between 7 and 217 ramets per m^2. As the lawns do not consist of large, uniform areas, but are cut in pieces for example in the rose garden, it is difficult to compute numbers of ramets using plant densities. However, in lawns where the species is present, the average density lies around 50 ramets per m^2, which is much higher than at the other locations. Locally, the density can even reach values of more than 2200 ramets per m^2! It can be estimated that the total number of ramets of *Apium repens* in the park reaches several hundreds of thousands.

These figures can be compared to those found by Lederbogen & al (2001) in Upper Bavaria, the region in Germany where the species has its main distribution. They mention average densities of 500 ramets per ha (0.05 per m^2) in large common meadows of each about 50 ha, with local densities up to 1000 ramets per m^2 in flooded grasslands. They conclude that *A. repens* displays an ecological optimum in flooded grasslands with permanent high groundwater levels, but is also important in periodically wet common meadows, that are extensively grazed. These would be adaptations of the species to the anthropogenically influenced land use, since originally, it is a species from tidal shores of lakes and river edges. It can rapidly colonize bare ground, but needs disturbed areas to survive, not being able to withstand the competition for light and nutrients of many other, common species. The very high densities that we found in the park lawns make it clear that *Apium repens* can thrive in yet other circumstances. The very high frequency of mowing of the lawns (every two weeks in the growing season) at a height of 4 cm appears to be an alternative form of disturbance that leads to optimal growing conditions, in combination with other conditions such as permanently high ground water levels.

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Population dynamics of the Polish Red Book Orchid *Neottianthe cucullata* (Orchidaceae) in NE Poland

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Abstract. *– A local population of* Neottianthe cucullata *in Wigierski National Park has been studied for five consecutive years in order to determine if there are symptoms of regression of this marginal population. It is one of few populations of this species existing in Poland. Individual plants grew in clumps of 10 – 200 on an area of about 1 ha. There were high fluctuations in the number of individuals (288 – 766) and in the fraction of juvenile and generative individuals (24 – 46% and 11 – 26% respectively), but no steady decrease over time was observed in number of individuals and in fraction of juvenile and generative plants. The mean number of flowers per inflorescence was positively correlated with the assimilation area of generative individuals and in subsequent years varied from 8 to 13. Number of flowers of individuals from the local population was smaller than in herbarium specimens. On the contrary their mean leaf area was larger. Differences in leaf area and number of flowers per inflorescence between flowering plants from studied population and herbarium specimens did not indicate a decline in plants' performance. Changes in population size, ratio of generative and juvenile plants, and biometric features don't give indications of population decline.*

Key words: *population dynamics,* Neottianthe cucullata*, terrestrial orchid.*

Introduction

Neottianthe cucullata (L.) Schlechter is a very small terrestrial orchid with one or two (seldom three) leaves and growing up to 30 cm high when flowering. It grows in pine and pine-spruce forests with well developed moss layer, close to water sources. It is the only European representative of eastern Asiatic genus *Neottianthe. N. cucullata* can be encountered from Japan and Sakhalin to Poland, where it reaches the SW limit of its geographical range (Filipek 1956, Hulten & Fries 1986). In Poland this species grows only at a few sites (Zajac & Zajac 2001). Like in neighbouring countries (Ukraine, Byelorussia, Lithuania and Kaliningrad District) it is very rare and considered an endangered species. It is mainly endangered by the loss of stands caused by forest clearings and natural succession (Kruszelnicki 2001, Piekos-Mirkowa & Mirek 2003).

Up to now only one study of three populations of this rare species has been carried out (Sarosiek & al. 1990). However, these authors have taken under consideration only flowering individuals. The present work is set as a beginning of long-term observations focused on the whole population including juvenile and vegetative individuals, to give insight into population dynamics of this species.

A preliminary three-year study suggested that the number of individuals decreases in time. The object of this study was to determine if this tendency continues for a longer period of time and

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to confirm if this local population of *N. cucullata* in Wigierski National Park in NE Poland is truly in regression stage.

Material and methods

In 1998, five permanent plots covering a total area of 166 m^2 were established in Wigierski National Park in NE Poland. Plots were located in an area with relative abundance of *N. cucullata* in order to estimate the condition of this local population. The established plots did not cover the area of the whole population in order to protect this site from possible unwanted influence caused by this study. Each year at the time of flowering, the number of individuals was recorded on all permanent plots. For each individual the number of leaves and their length and width was noted. For flowering individuals also the number of flowers was recorded. All individuals were classified to one of three groups according to number of basal leaves, and absence or presence of inflorescence: 1 generative – flowering individuals, or individuals with underdeveloped or damaged but visible inflorescence, and with two or three leaves; 2 vegetative – plants with two or three leaves but lacking the inflorescence; 3 juvenile – plants with only one leaf and without inflorescence.

The height of individual is not a good estimate of *N. cucullata* plant's size. Non-flowering plants have only one or two (seldom three) basal leaves which lay flat on the surface of soil. Plant size was therefore estimated from the area of leaves approximated by the ellipse equation from length and width of leaves, as this more precisely reflects the size and performance of individual plants.

The size of leaf area and number of flowers per inflorescence was also recorded for about 100 herbarium specimens from the herbaria of Turk, Göteborg, Uppsala, and Stockholm, from different locations of species range from Poland, Lithuania, former Soviet Union, Nepal, China and Japan. This data was then compared with generative individuals from local population in order to assess the local plants' performance.

Results

Over five years study high fluctuations and no steady decrease in number of individuals was observed. During the first three years the number of individuals decreased reaching the lowest level (about 60% of individuals recorded in 1998) in 2000. In the next year the highest number of plants was observed (over 2.5 times the number of individuals recorded in 2000). The year 2001 was followed by a downfall of number of individuals in 2002, which still was higher than population size observed in the first three years of this study (fig. 1).

Similarly the fraction of juvenile individuals did not decrease steadily over time. It was highest in the first year of this study, when young plants constituted almost 50% of all individuals. In the next year the fraction of juveniles decreased rising again in 2000. In 2001 the fraction of young plants reached 35 %. The year 2002 brought a further minor increase in the fraction of plants in the juvenile stage (fig. 1, tab. 1). Thus there was no indication of recruitment limitation in this population.

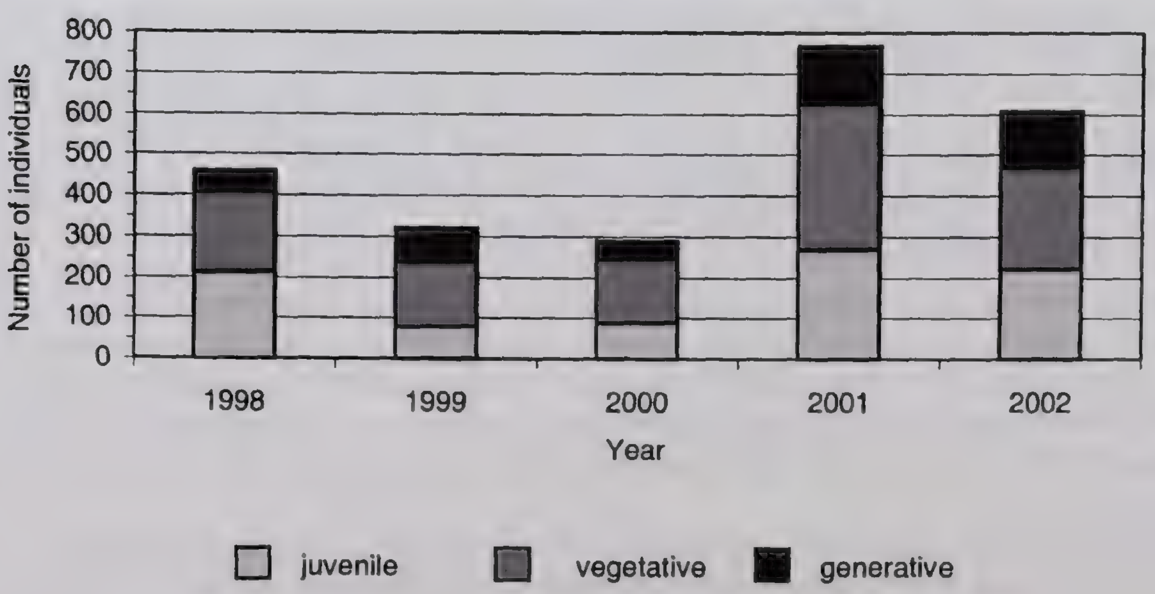

Figure 1. Number of individuals in all developmental stages in consecutive years.

Also the fraction of generative plants did not drop over the five years of this study. The percent of generative individuals was lower and more stable than the fraction of juveniles. In 1998 plants in the generative stage constituted only about 10% of all individuals. In the following year the fraction increased to over a quarter and in 2000 it fell to 13 %. In the next two years the fraction of generative plants slowly increased reaching over 20% in 2002 (fig. 1, tab. 1).

Table 1. Fraction of juvenile, vegetative and generative individuals in consecutive years.

Year	Juvenile	Vegetative	Generative
1998	46	43	11
1999	24	50	26
2000	31	56	13
2001	35	47	18
2002	37	41	22

Not all plants in the generative stage possessed a well developed inflorescence, therefore not all individuals in this developmental stage had influence on fruit set in this population. The fraction of flowering individuals with undamaged inflorescence varied from only 5% and twenty-four individuals in 1998 to 14% and a maximum of 111 individuals in 2001. In 1999 fifty-one flowering individuals constituted 16% of all studied plants. In 2000 there were twenty-nine flowering plants and in 2002 eighty-five (10% and 14% respectively).

The fraction of vegetative plants was much more stable varying only from 41 % to 56 % (fig. 1, tab. 1).

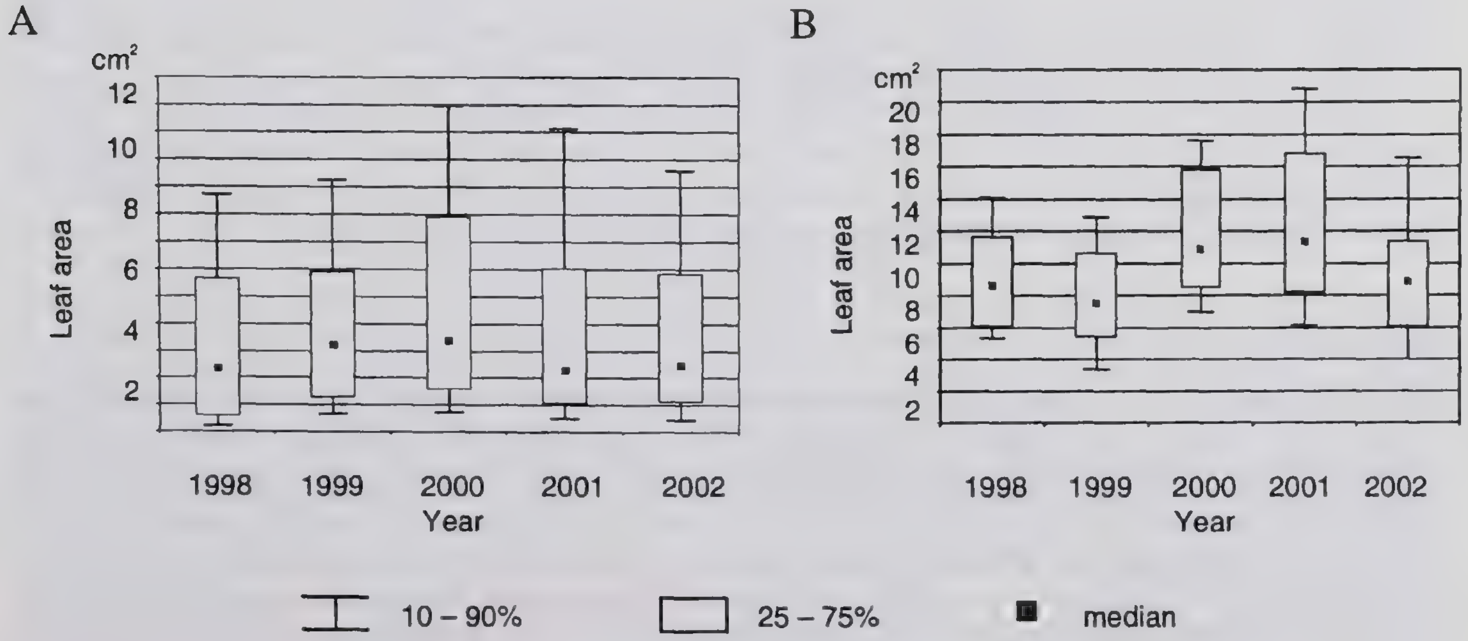

Figure 2. Size of leaf area (cm²) in consecutive years.
A, all individuals; B, generative individuals.

No steady decrease in plant size (leaf area) was observed. The mean leaf area of all individuals oscillated between 3.57 cm² and 5.09 cm² (fig. 2A). Plants in generative stage were larger than all other individuals. Their mean leaf area fluctuated between 8.46 cm^2 and 12.88 cm^2 (fig. 2B) and was considerably higher (Mann-Whitney U test $p<0.01$) than the mean leaf area of plants in vegetative and juvenile developmental stages. This suggests that *N. cucullata*, in order to reproduce, needs to reach a minimum size of leaf area, which is responsible for collecting resources for flowering and seed production.

The mean number of flowers per inflorescence oscillated between eight and ten with an exception of the year 2001, when it reached a maximum of thirteen flowers per flowering individual (fig. 3). The number of flowers was positively correlated with the assimilation area of generative individuals (Spearman $R=0.599$, $N=191$, $p<0.0001$).

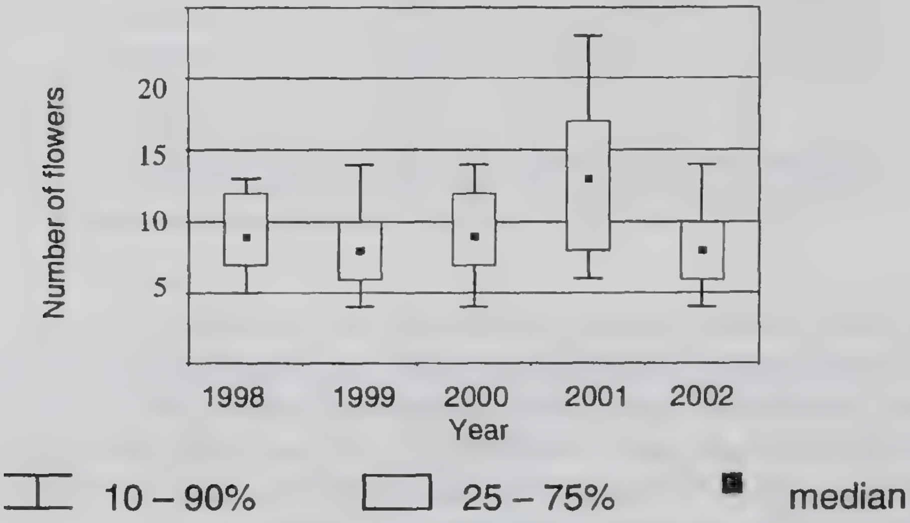

Figure 3. Number of flowers per inflorescence in consecutive years.

Both for local flowering plants and herbarium specimens the highest fraction of individuals had leaf area of about 9 cm^2 and six to ten flowers per inflorescence.

The leaf area of generative individuals from local population was slightly larger than of herbarium specimens (median 9.82 cm^2 and 8.41 cm^2 respectively, Mann-Whitney U test $p<0.02$), but herbarium specimens had more flowers per inflorescence than local population flowering plants (median 11.93 and 10.35 respectively, Mann-Whitney U test $p<0.02$).

Discussion

The variation of size of the local population was considerably smaller than ten to fifteen fold changes reported in literature (Wisniewski 1975). The observed maximum size of the local population was only 2.5 times larger than the minimum. This difference may be caused by a too short series of observations or by taking into account that Filipek (1956) and Wisniewski (1975) only considered flowering individuals and neglected the influence of non flowering plants on the population size. Fluctuations in number of flowering individuals in the local population of 4.5 fold are closer to those cited in the literature, but still more than twice smaller. This might indicate that the studied population is rather stable or that the observations have been carried out for a too short a period of time.

The appearance of juvenile and generative plants was recorded each year with different quantities and no steady downfall in the fraction of juvenile and generative individuals was observed. The flowers of *N. cucullata* are pollinated and generative plants produce seeds

(Wodkiewicz pers. obs.). No germination tests have been carried out. Thus no limitation of recruitment of new individuals by lack of seed production and their germination was observed. Observations of appearance of new individuals may lead to the conclusion that conditions for flowering, seed dispersal and recruitment of new individuals are favourable. The observed fluctuations might be caused by different environmental conditions associated with the weather. In the year 2001 the highest number of all individuals, highest number of flowers per inflorescence and the largest leaf area of generative individuals was observed. Also moderately high fractions of generative and juvenile plants were noted. This might be caused by high mean and minimum temperature together with relatively high precipitation in July 2001. Perhaps flowering of *N. cucullata* is also promoted, as in other orchid species (Wells & al. 1998), by good performance of plants in the previous year as the largest leaf area of all individuals was observed in 2000.

The higher number of flowers in herbarium specimens may be caused by a manner of collecting plants for herbarium. Herbarium specimens are not a random sample of plants from different populations because collectors tend rather to pick well developed and nice specimens. Young and non-flowering plants were therefore excluded from the comparison of local plants with herbarium specimens. The overall comparison did not indicate a poorer performance of plants from Wigierski National Park. It must also be noted, that a high number of flowers comparable with herbarium specimens has been noted in 2001 proving a capability of this marginal population to produce, under favourable conditions, as many flowers per inflorescence as in other populations in the centre of the species range. Also the results of comparison of leaf area confirm that performance of plants from this local population is not worse than in other populations.

The results seem to suggest that the local population is not in regression stage, however a much longer series of observations is needed to confirm this. Hopefully this population under the protection of Wigierski National Park will be safe from the main threat of extinction of this species, which is the destruction of sites associated with forest management.

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Cochlearia macrorrhiza, a highly endangered lowland species from Eastern Austria. Conservation genetics, *ex situ* and *in situ* conservation efforts

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Abstract. *– The highly endangered endemic* Cochlearia macrorrhiza *(Brassicaceae) is currently represented by two individuals only at its natural stand in Moosbrunn near Vienna, Austria. A second sub-population has been cultivated in Berlin for nearly two decades. Therefore, a research and conservation programme has been initiated to measure genetic variation and fitness parameters of plants from both subpopulations in order to (1) confirm the authentic origin of the material from Berlin, (2) to evaluate the taxonomic status of* C. macrorrhiza, *and (3) to detect genetic markers distinguishing the gene pools of both subpopulations for future breeding programs. In parallel, phenotypic characters and fitness parameters have been measured to test for significant differences between subpopulations. The aim is to establish a large population sampling for* ex situ *cultivation, to build up an* ex situ seed bank, and to start a re-introduction experiment.

Key words: Cochlearia macrorrhiza, *Brassicaceae, conservation genetics, species conservation,* ex situ *conservation,* in situ *conservation.*

Introduction

Cochlearia macrorrhiza is one of the most highly endangered species in Central Europe. Two individuals only survived at its natural stand in a lowland area between the Eastern Alps and the Carpathians in Austria approximately, 25 km southeast of Vienna. Molecular studies using amplified fragment length polymorphisms (AFLPs) and isozyme analysis confirmed the status of *C. macrorrhiza* as a distinct taxon (Koch 2002, Koch & al. 2003). However, it was shown that lowland *C. macrorrhiza* does not genetically bridge the distribution area of montane and alpine *Cochlearia* species from the Eastern Alps and the Carpathians (Koch & al. 2003). *Cochlearia macrorrhiza* represents a separate lineage, which evolved from a diploid *Cochlearia* ancestor, presumably *C. pyrenaica*, as *C. excelsa* did in East Austrian high alpine regions (Koch & al. 2003). *Cochlearia macrorrhiza* is self-compatible, a biological character that is shared with the other diploid *Cochlearia* species (Koch & al. 1998).

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However, in diploid *Cochlearia* flower architecture maintain high levels of outbreeding and natural populations are normally found to be in Hardy-Weinberg equilibrium (Koch 2002, Koch & al. 2003).

The species is adapted to undisturbed habitats characterised by permanently flooding cold water with high carbonate concentrations and needs open places with reduced competition in the corresponding fen vegetation. This vegetation is characterised by rare species such as *Primula farinosa*, *Cladium mariscus*, *Schoenus nigricans*, or *Gladiolus palustris* (Höfner 1987, Korner & al. 1998-2002). Many of them are highly endangered in Austria, and are listed in most Central European red data books of vascular plant species. This habitat is protected by the austrian government and belongs to the endangered habits listet in the european FFH-directive (fauna-flora-habitat-directive).

Lowland fens are characteristic habitats and contribute to important vegetation complexes of the higher and lower Alps. Their existence always depends on high ground water levels. During the last decades drainage and intensified agricultural activities have led to an enormous loss of these habitats, and many lowland moor sites have gone extinct or have been reduced to small, fragmentary relictual areas (Steiner 1992). As a consequence, units of vegetation have disappeared or are highly endangered. With these units of vegetation many characteristic plant species of the lowland moors are endangered, too. This situation also holds true for the habitat of *C. macrorrhiza* near Moosbrunn.

Remarkable for conservation efforts is the fact that no soil seed bank is established by this species (Koch, unpublished results). Similar results have been obtained for the closely related *C. pyrenaica* (Abs 2002), the montane sister taxon, which is also highly endangered.

In the early 1980's several individuals of *C. macrorrhiza* have been transferred from its natural stand to Berlin, Botanical Garden Berlin-Dahlem, and are still under cultivation. The current population size reaches several dozens individuals.

The central aim of this study was to establish and document an *ex situ* collection of *C. macrorrhiza* for future *in situ* experiments and conservation efforts. However, in the context of this study we also asked the question about the consequences of "uncontrolled" or "undocumented" propagation (vegetative as well as generative) in a Botanical Garden over a time period of twenty years and its influence on genetic diversity and fitness parameters compared to the situation at its natural stand, where population size declined rapidly throughout the last decades.

Material and methods

We obtained seed material from a bulked sample from Berlin (Botanical Garden Berlin-Dahlem, approximately 250 seeds) and seeds (collected in 2000) from the last two mother plants of *C. macrorrhiza* at its natural stand near Vienna in Austria (approximatly 100 seeds from each plant). At this time we found only three additional seedlings at the natural site resulting in a total population size of five individuals.

Seeds of both subpopulations and some control material from *C. pyrenaica*, *C. tatrae* were weighted. We also collected seed material from *C. macrorrhiza* at Moosbrunn in 1999 and 2001 to analyse year-by-year seed weight variation. Germination tests from both collections (Berlin and Moosbrunn) of the year 2000 were performed on wet filter paper in petri dishes under the following temperature regime using the total seed material: 1 day at room temperature (dark), 5 days at 4°C (dark), from the 6th day on 12 hours at room temperature/12 hours at 10°C (dark) for three weeks.

Subsequently, we cultivated 17 seedlings from Berlin and 20 seedlings from each mother plant from Moosbrunn in the greenhouse. In total, our analysis started with 57 F1-individuals.

All plants were grown for one year in the greenhouse to obtain healthy plants cultivated under optimal growing conditions. In autumn 2001 we transferred all individuals from the greenhouse and started a common garden experiment in Vienna. *Cochlearia macrorrhiza* requires vernalization for optimal flowering and it was necessary to keep the plants outside during the winter. In spring 2002 we scored flowering time measured as opening time of the first flower bud using high alpine *C. excelsa* as control. Additionally we scored the number of branches (first order) within the inflorescence, the total number of fruits with fertile and sterile/aborted seeds, and the F2 seed weight. Cross-pollination was possible within the two subpopulations Berlin and Moosbrunn, respectively, but not between them.

Individual genetic variation and differentiation have been analysed using AFLPs (amplified fragment length polymorphism). Detailed protocols and the corresponding primary results have been already reported (Koch et al. 2003).

For the present study the same binary (0/1) data matrix (presence and absence of particular AFLP bands) was used to perform a principal coordinate analysis (3-dimensional) (Podani 1993), resulting for each indiviudal in three coordinates characterising its genetic differentiation. These three data sets were used for subsequent stastistical analysis using SPSS ver. 10.07.

Statistical analyses were performed using SPSS. From the primary data we also estimated "fertility" as the number of fertile fruits divided by the number of sterile fruits. Data were obtained from all 57 individuals. After confirming normal distribution of these data (Kolmogorov-Smirnov-test, implemented in SPSS) we tested for differences between (1) the two offspring families from Moosbrunn (each 20 individuals in size), and (2) between the 17 individuals from Berlin and the 40 individuals from Moosbrunn using one-way ANOVA (as implemented in SPSS).

Results

The data of the germination experiment are shown in fig. 1. The germination rate for the seed material from Berlin was much higher (88%) than for the material from Moosbrunn (21%). Similarly, seed weight was higher for the original seed material from Berlin than for the corresponding seed material collected in 2000 at Moosbrunn (table 1). However, there were no differences in seed weight when comparing the F2 seed material collected in 2002 (table 1).

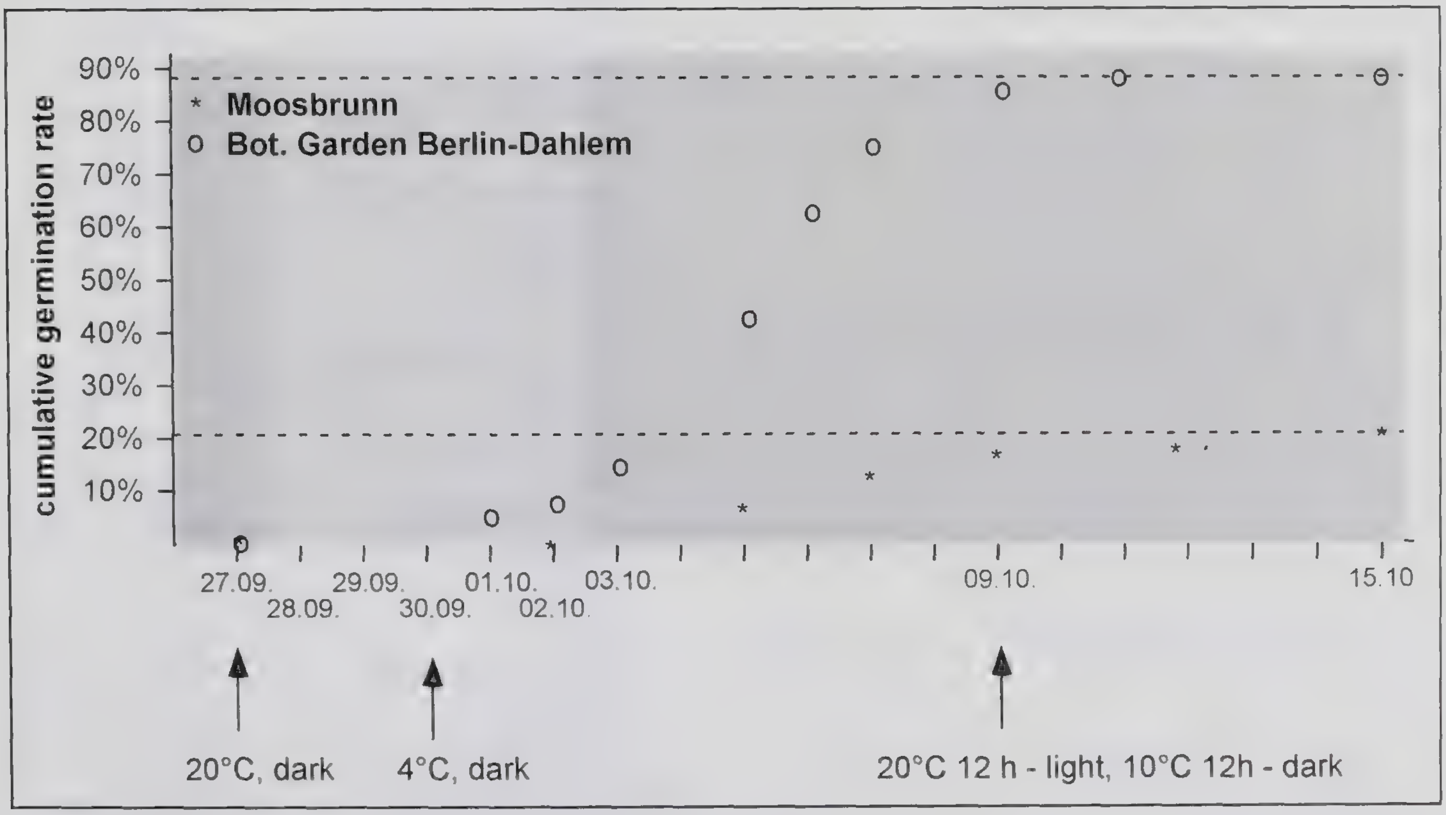

Figure 1. Germination rates of the Cochlearia macrorrhiza *seed material from Berlin and Moosbrunn.*

Table 1. Seed weight (100-seed-weight in gr) and ploidy levels of *Cochlearia macrorrhiza* and some control species. For population details refer to Koch & al. (2003).

C. macrorrhiza	Berlin	0.1700	diploid
	Berlin F2 2002	0.1011	diploid
	Moosbrunn 1999	0.1218	diploid
	Moosbrunn 2000	0.1440	diploid
	Moosbrunn 2001	0.1368	diploid
	Moosbrunn F2 2002	0.1129	diploid
C. pyrenaica ssp.*borsaeana* (Romania)		0.0996	hexaploid
C. pyrenaica (Annaberg, Austria)		0.1175	diploid
C. tatrae (Nohavica, Slovakia)		0.0767	hexaploid
C. tatrae (Veleicka, Slovakia)		0.1027	hexaploid

Table 2. Summarized descriptive statistics of *Cochlearia macrorrhiza* from (1) Berlin and (2) Moosbrunn.
* Relative flowering time, days after the first flower of *C. excelsa* appeared.

Character set	Origin		
1 – branches in inflorescence	1	Mean	9.64
		Standard deviation	2.27
		Minimum	4
		Maximum	14
	2	Mean	6.18
		Standard deviation	2.32
		Minimum	3
		Maximum	10
2 - sterile fruits (per plant)	1	Mean	293.11
		Standard deviation	120.79
		Minimum	118
		Maximum	576
	2	Mean	178.00
		Standard deviation	138.88
		Minimum	58
		Maximum	547
3 - fertile fruits (per plant)	1	Mean	85.58
		Standard deviation	40.80
		Minimum	25
		Maximum	187
	2	Mean	43.09
		Standard deviation	25.77
		Minimum	5
		Maximum	83
4 - quotient: 2/3 ("fertility")	1	Mean	0.29
		Standard deviation	0.078
		Minimum	0.114
		Maximum	0.451
	2	Mean	0.37
		Standard deviation	0.303
		Minimum	0.016
		Maximum	0.897
5 – relative flowering time *	1	Mean	8.86
		Standard deviation	4.13
		Minimum	1
		Maximum	17
	2	Mean	15.36
		Standard deviation	5.84
		Minimum	11
		Maximum	32
6 – seed weight F2	1	Mean	0.1129
		Standard deviation	0.0235
		Minimum	0.0500
		Maximum	0.1711
	2	Mean	0.1011
		Standard deviation	0.0330
		Minimum	0.0457
		Maximum	0.1457

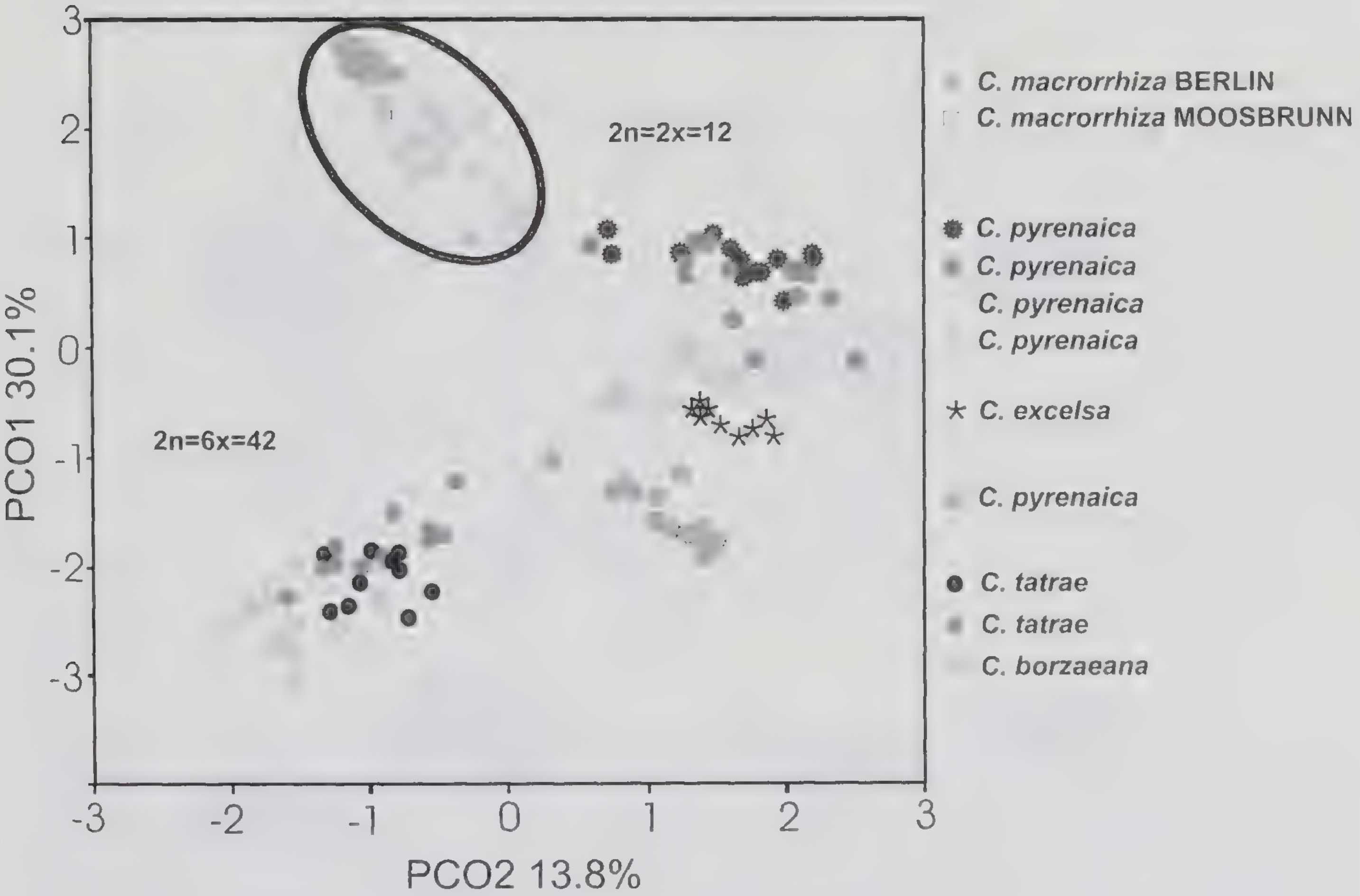

Figure 2. Redrawn Principal Coordinate Analysis (PCA) of Cochlearia *species from Koch & al. (2003).*

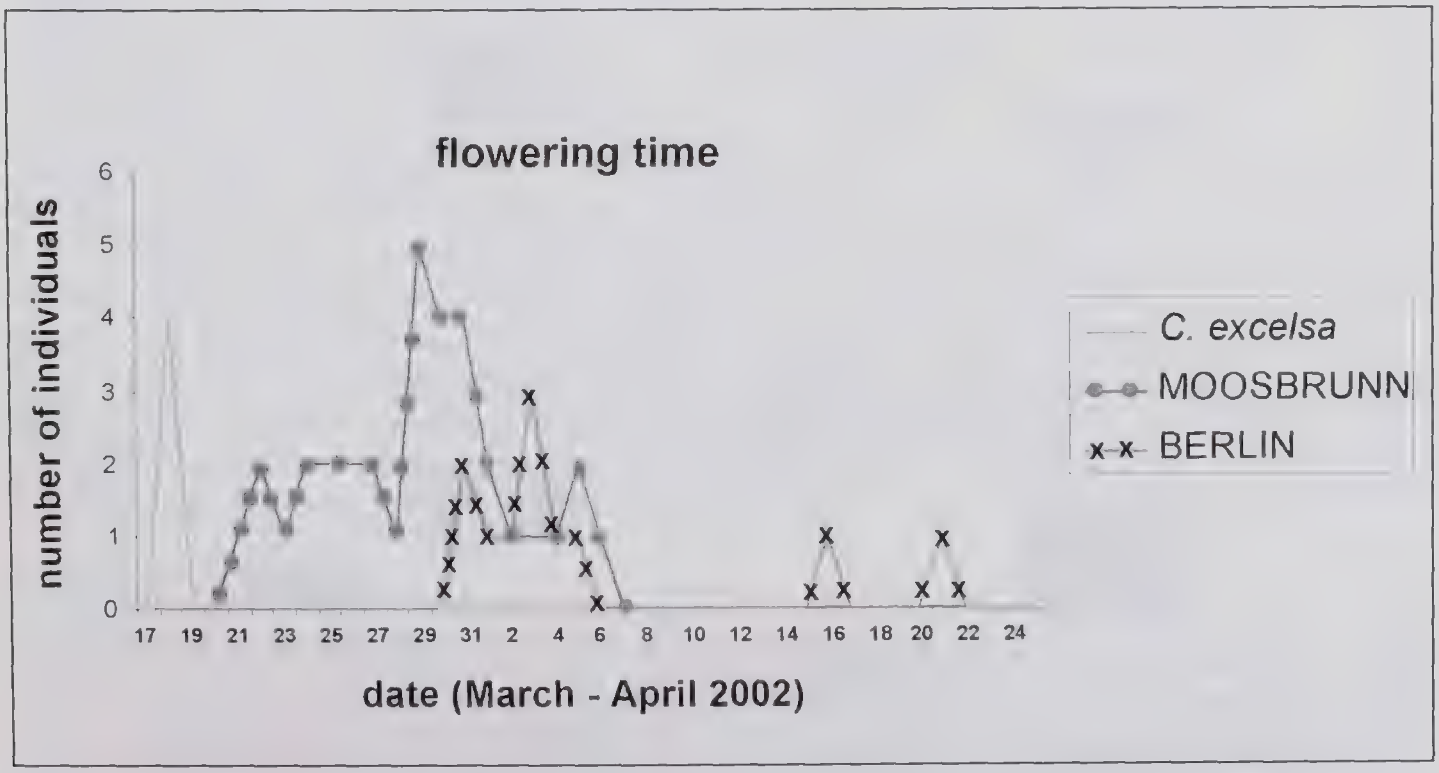

Figure 3. Flowering time of the two subpopulations in 2002.
Cochlearia excelsa*, a high-alpine species, has been used as internal control.*

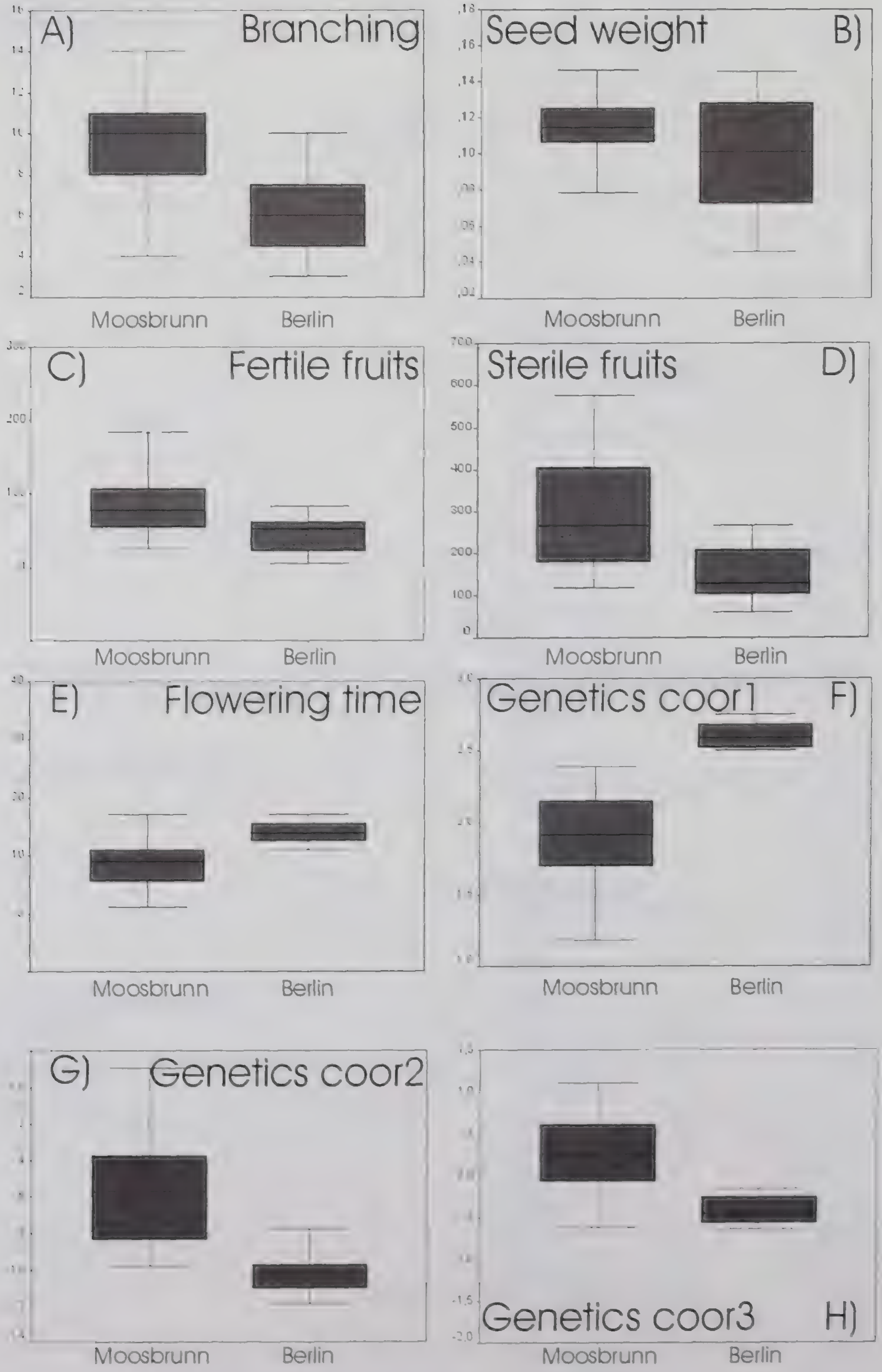

Figure 4. Boxplot diagrams for several characters measured for Cochlearia macrorrhiza *subpopulations. For the genetic differentiation the first three coordinates from the PCA have been choosen (see fig. 2).*

Table 3. Correlation among the different characters under study (according to Pearson) and its significance (2-tailed).

		1	2	3	4	5	6	7	8
1) Seed weight	Correlation		0.247	__-0.413**__	-0.193	__0.302**__	0.000	-0.150	0.051
	Significance		0.094	0.004	0.193	0.039	1.000	0.397	0.773
2) branching	Correlation	0.247		__-0.524**__	__-0.549**__	-0.251	__-0.440**__	__0.153**__	__0.341*__
	Significance	0.094		0.000	0.000	0.089	0.009	0.386	0.048
3) flowering time	Correlation	__-0.413**__	__-0.524**__		__0.525**__	0.002	__0.420*__	-0.226	-0.289
	Significance	0.004	0.000		0.000	0.988	0.013	0.198	0.097
4) origin	Correlation	-0.193	__-0.549**__	__0.525**__		0.214	__0.798**__	__-0.557**__	__-0.681**__
	Significance	0.193	0.000	0.000		0.148	0.000	0.000	0.000
5) sterile/fertile fruits	Correlation	__0.302*__	-0.251	0.002	0.214		0.175	-0.115	-0.060
	Significance	0.039	0.089	0.988	0.148		0.321	0.517	0.738
6) genetics coor.1	Correlation	0.000	__-0.440**__	__0.420*__	__0.798**__	0.175		__-0.653**__	__-0.880**__
	Significance	1.000	0.009	0.013	0.000	0.321		0.000	0.000
7) genetics coor.2	Correlation	-0.150	__0.153**__	-0.226	__-0.557**__	-0.115	__-0.653**__		__0.442**__
	Significance	0.397	0.386	0.198	0.000	0.517	0.000		0.004
8) genetics coor.3	Correlation	0.051	__0.341*__	-0.289	__-0.681**__	-0.060	__-0.880**__	__0.442**__	
	Significance	0.773	0.048	0.097	0.000	0.738	0.000	0.004	

__** $p \leq 0.01$__
__* $p \leq 0.05$__

The results of the principal coordinate analysis of molecular differentiation as revealed by AFLPs were redrawn from Koch & al. (2003) and in fig. 2 the first two coordinates are shown. These data demonstrate that (1) *C. macrorrhiza* is genetically distinct from other diploid *Cochlearia*, and (2) both gene pools, Berlin and Moosbrunn, are significantly differentiated from each other. Considering these two gene pools, among the 68 variable AFLP fragments we detected only eight and five AFLP fragments characteristic for Berlin (63 variable AFLP bands) and Moosbrunn (60 variable AFLP bands), respectively.

Comparisons of the different data sets (morphology, phenological traits, fitness and genetic parameters) between the two groups of twenty individuals each from Moosbrunn revealed no significant differences and the results are not shown here. The descriptive statistics of both subpopulations, Berlin and Moosbrunn, are summarised in table 2.

Significant differences (ANOVA) between subpopulations were found in (1) flowering time ($p = 0.001$) (fig. 3), (2) number of branches in the inflorescences ($p = 0.001$), (3) genetic differentiation [for all three coordinates separately; and in total explaining more than 53% of the total genetic variation] ($p = 0.001$, each), (4) number of fertile fruits ($p = 0.002$), and (5) number of sterile fruits ($p = 0.018$). No significant differences were found in 1) F2 seed weight ($p = 0.193$), and 2) "fertility" (number of fertile fruits divided by number sterile fruits) ($p = 0.302$). The corresponding boxplot diagrams are shown in fig. 4. In order to test correlations among the different data sets we performed a correlation test according to Pearson. The results are shown in table 3 and demonstrate some additional highly significant correlation of seed-weight with "fertility", decreased flowering time with increased seed weight, and increased flowering time with increased inflorescence branching.

Discussion

Molecular/genetical differentiation. One important conclusion from our molecular data is that we got no evidence that other taxa than *C. macrorrhiza* contributed to the gene pools we analysed. This is even true for the material from Berlin, where other *Cochlearia* taxa have been cultivated also during the time period of twenty years. In sharp contrast, however, is the decrease of genetic variation in the Berlin population compared to the Moosbrunn population. Although only two single individuals remained at its last natural stand this population was able to maintain higher levels of genetic variation than the much larger population from Berlin did. The reason for this is unclear, but the most likely explanation is increased inbreeding due to isolation (either simply by distance or, more likely, by lacking effective pollinators). Consequently, genetic drift led to significantly decreased levels of genetic variation.

Phenotypic variation. Similar to the molecular data phenotypic variation is always higher in Moosbrunn than in Berlin (fig. 4). The explanation for this should be the same as for the genetical variation as explained obove. However, the consequences are more far-reaching, because here we have a direct effect of phenotypic traits that might also effect fitness of the plant.

Outlook. Our most important conclusion is that the *ex situ* cultivated population in Berlin exhibit lower variation in most traits under study than the natural population from Moosbrunn, which was represented in 2001 by two flowering individuals only. Taking also the genetic data into account it is obvious that the *in situ* Moosbrunn population was affected by inbreeding and genetic drift to a much lower extent than the *ex situ* collection in Berlin. However, phenotypic and genetic plasticity is a prerequisite for future conservation efforts. In particular, seedling establishment is one of the most critical phases in the life cycle of *C. macrorrhiza* and, therefore, colonisation success greatly depends on a sufficiently high number of viable seeds produced by the mother plants.

Therefore, our results demonstrate the need of maintaining highest genetic variation in *in situ* collections, if such collections should serve as a resource for further protection programs.

For our future studies this initial screening demonstrates that both gene pools should be considered. Consequently, we will begin to cross both of them to produce a larger collection of a "joint" population. This will be accompanied by the same measurements of single offspring plants as presented in this paper.

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Saving wild African violets (*Saintpaulia*, Gesneriaceae): a review of ongoing activities and a plan for *ex situ* conservation

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Abstract. – *African violets (*Saintpaulia *H.Wendl., Gesneriaceae) are among the world's most popular houseplants. Thousands of cultivars exist and are commercially grown for homes and offices throughout Europe and North America. The annual wholesale value of* Saintpaulia *in the USA and the Netherlands alone is over € 50 million. In contrast to the popularity and economic value of the cultivars, the native distribution of the genus is restricted to small areas in Tanzania and Kenya, and most of the c. 25 wild species are endangered. Urgent actions are needed to save the genetic diversity of the genus. However, basic information on the distribution, habitat requirements, and accurate conservation status of the wild species is inadequate, and very few data exist on their biology, which hampers effective conservation work. In the last decade, the growing awareness among botanists and conservationists of the critical situation of* Saintpaulia *has led to a number of research and conservation initiatives. This paper provides an overview of the activities known to the authors: research on systematics, phylogenetics, population biology, and conservation biology, and* in situ *and* ex situ *conservation initiatives. The ongoing activities are placed against a listing of needs of information and measures for efficient conservation of the genus. Critical information and action gaps are identified. Plans to fill one of the gaps —* ex situ *conservation in European botanic gardens — are put forward. A preliminary listing of* Saintpaulia *accessions in some European botanic gardens is presented, and a call for collaboration in* ex situ *conservation is issued.*

Key words: *Botanic Gardens, Eastern Arc mountains,* in situ *conservation, Kenya, Tanzania.*

Introduction

African violets (*Saintpaulia* H.Wendl., Gesneriaceae) are small rosulate or procumbent perennial herbs with hairy leaves. They have lovely violet-blue to whitish flowers and are comparatively easy to grow and propagate. These characteristics have placed them among the most popular houseplants in the world. Since the introduction of two species to Europe in the late 19th century, thousands of cultivars have been developed and commercially grown for innumerable homes and offices throughout Europe and North America (Baatvik 1993; see also http://www.avsa.org/). The annual wholesale value of *Saintpaulia* cultivars in the USA and the Netherlands, two leading horticultural producers, amounts to over € 50 million (Anonymous 1999a, b). This places them also among the economically most important potted plants.

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In the wild, there are twenty described species and four varieties of *Saintpaulia* plus perhaps half a dozen as yet undescribed species (Baatvik 1993; Eastwood & al. 1998; Watkins & al. 2002). All are endemic to Kenya (3 or 4 spp.) or Tanzania (the remaining taxa) in tropical East Africa. The genus has been promoted as a flagship taxon, 'the giant panda' of East African plant conservation, because it is well known and popular, and because the wild taxa are endangered (Baatvik 1993; Simiyu & al. 1996; Eastwood & al. 1998; Walter & Gillet 1998). It is clear that actions are needed to protect the genetic diversity of the genus. However, despite recently invigorated interest in African violets among scientists and conservationists alike, available data are still insufficient to plan and execute effective conservation measures.

In this paper we start with briefly reviewing the available information on *Saintpaulia*. We then give an overview of research and conservation activities on the genus, which we know have been or are being carried out. Our aim is to identify gaps in the available information and ongoing efforts, which need to be bridged if we wish to save the genetic diversity of this horticulturally valuable group. Plans for eliminating one of the identified gaps, *ex situ* conservation in botanic gardens, are put forward in the last section of the paper.

Conservation status and needs

In addition to being endemic to a restricted geographical region, the species of *Saintpaulia* typically have a patchy distribution. Most species are confined to rainforests growing on the slopes of the so-called Eastern Arc Mountains, a chain of ancient crystalline mountain blocks running from southern Kenya through the eastern parts of Tanzania, while a few are found in the adjacent coastal lowlands. Furthermore, most species occur only in one or a few of the isolated massifs (Burtt 1958; Simiyu & al. 1996; Eastwood & al. 1998; Watkins & al. 2002).

African violets also have specific habitat requirements, which further contributes to a fragmented spatial structure of the populations (Johansson 1978; Baatvik 1993; Simiyu & al. 1996). They seem to be weak competitors, which require moist, shaded conditions (Johansson 1978; Baatvik 1993; Eastwood & al. 1998). The plants typically grow on almost bare rock outcrops along forest brooks, although they are sometimes also found on the forest floor and even on tree trunks.

Due to the restricted distribution and special habitat requirements, and to anthropogenic pressure on the populations and their habitats, most if not all species of *Saintpaulia* are endangered. However, it is illustrative of the level of scientific knowledge about the genus that of the 20 taxa listed in the 1997 IUCN Red List (Walter & Gillet 1998), the status of 16 was categorized as 'Indeterminate'. Subsequently, in the most comprehensive review to date, Eastwood & al. (1998) used "all the available current information ... from literature, botanic garden databases, and the authors' own field experience" to assess the conservation status of *Saintpaulia*. They assigned new categories of threat (*sensu* IUCN 1994) to 28 *Saintpaulia* taxa. They deemed it possible to assign three taxa the category 'Vulnerable' and 16 the category 'Critical', although there were still nine taxa that could not be reliably appraised (category 'Data Deficient').

Ongoing research and conservation activities

It is noteworthy that in the thorough review of Eastwood & al. (1998) no surveys of population sizes or dynamics were cited. This is because, to our knowledge, no such information has been published on African violets, despite their fame and popularity. The study of the wild species has so far concentrated on their taxonomy (e.g. Burtt 1958, 1964), floristics (Watkins & al. 2002), and phylogenetics (Möller & Cronk 1997a, b, 2001; Lindqvist & Albert 1999, 2001). Only sporadic observations have been made on the ecology of the wild species (Johansson 1978; Simiyu & al. 1996), although horticultural accounts on cultivars abound.

More information on the ecology and biology of the wild populations is to be expected shortly. At the University of Helsinki, a series of studies are being conducted on wild populations in the East Usambara Mountains, Tanzania. Preliminary results on the population ecology of four species already exist (Kolehmainen 2000). More data are currently gathered by J. Kolehmainen and students on the population dynamics in relation to variation in abiotic parameters, on the pollination biology, and on the population biology of a number of taxa. Mapping of the distribution of East Usambaran species down to the population level has also commenced, and these activities should soon extend to other Tanzanian mountain ranges as well (the Saintpaulia Mapping and Monitoring Project SMMP, University of Helsinki). This work also includes assessment of threats to the populations. Knowledge on the distribution and conservation status of the Kenyan taxa is already better (Simiyu & al. 1996).

In situ conservation of the East Usambaran taxa took a considerable step forward with the establishment of Amani Nature Reserve in 1997 (Anonymous 1997a, b). Efforts are in progress to achieve *in situ* protection of Kenyan taxa (Simiyu & al. 1996). Building of awareness among conservation authorities and local inhabitants in Tanzania is included in the goals of the SMMP. Even so, most species are not protected *in situ*, and most probably will not be in the immediate future. Controlled *ex situ* conservation of Kenyan taxa is well under way at the East African Herbarium in Kenya (Simiyu & al. 1996). A few Tanzanian taxa are also included, and some exist in *ex situ* collections in Tanzania (Amani & Dar es Salaam; Eastwood & al. 1998).

Information and action gaps

Lack of fundamental-scientific information seriously hampers the design of appropriate conservation measures for *Saintpaulia*. The problems include deficient taxonomy (Simiyu & al. 1996), uncertainties as regards phylogenetic history and ancestral areas (Möller & Cronk 1997a; Lindqvist & Albert 1999, 2001), insufficient understanding on the distribution of most of the taxa (see, e.g. Baatvik 1993), and lack of information on gene exhange and genetic variation among populations, on relative roles of sexual vs. asexual reproduction, on dispersal and pollination mechanisms, as well as on population dynamics in relation to abiotic variables. These all are data that are needed to plan the selection of accessions for *ex situ* conservation collections and to target the most critical populations with the right means in *in situ* conservation. In addition, applied studies on the threats to wild populations, on their conservation status, on the economic value of wild species in the horticultural trade, and on their potential role in attracting eco-tourism are needed for the practical planning of conservation measures. Information on these subjects would also be valuable for promoting *Saintpaulia* conservation among decision-makers, funding agencies, and the general public.

Improving the knowledge base is, of course, not enough for ensuring the continued survival of species of *Saintpaulia* in the wild. If habitat destruction continues, as it likely will, current *in situ* conservation measures are not sufficient for saving even the majority of the genetic diversity of the genus. It seems unlikely that most taxa could be protected by new conservation areas in the near future. Therefore, in the case of *Saintpaulia*, *ex situ* conservation is a necessary complement to *in situ* measures. Current activities in this field, although laudable *per se*, may not be enough for securing the existence of this horticulturally valuable gene pool. Furthermore, since current activites are located in Kenya and Tanzania (which in itself is perfectly appropriate), the work does not benefit from the publicity and, consequently, increased financial support it could attract in the wealthy north. For these reasons we feel well-established European botanic gardens should take a more active role in the conservation of African violets.

Ex situ conservation: the role of European botanic gardens

A considerable number of *Saintpaulia* accessions of known wild origin are already held by a number of European botanic gardens and by some private collectors, and all but three taxa are included (table 1). However, at present these function only as passive repositories of *Saintpaulia* germplasm since they are not developed with any explicit goals as regards the conservation of the genetic diversity of the genus. Furthermore, no systematic cross-referencing of the collections has been carried out, which means that the material of some taxa represented in several gardens may actually derive from one single plant that has been propagated by leaf cuttings, i.e., vegetatively cloned. Due to the problematic taxonomy of parts of the genus (e.g. Burtt 1964, Lindqvist & Albert 1999), the species identifications of the *ex situ* collections should also be cross-checked and homogenised.

Table 1. ***Ex situ*** **holdings of** ***Saintpaulia*** **in major botanic garden collections in Europe (shaded columns), in three African collections, and in those of some private collectors.**

The information has been gathered from the following sources: Eastwood & al. (1998), Watkins & al. (2002), and the database of Helsinki University Botanic Garden (available at: http://www.helsinki.fi/ml/botgard/index-e.html). Note that no attempt has been made to verify or update the information concerning other gardens or private collections. v. = variety, sp. n. = new undescribed species, Upp. = Uppsala, Ed. = Edinburgh, Mei. = Meise, Hki = Helsinki, Bas. = Basel, Nai. = Nairobi, DeS = Dar es Salaam, Ama = Amani, tot. = total, gdns. = gardens, ps. = Private

	TAXON	Upp.	Ed.	Mei.	Hki	Kew	Bas.	Nai.	DeS	Ama	tot. gdns.	pr.
1	*S. brevipilosa*	X	X	X	X	X		X			6	X
2	*S. confusa*	X	X	X	X	X	X	X	X	X	9	X
3	*S. difficilis*	X	X	X	X	X		X	X	X	8	X
4	*S. diplotricha*	X	X	X	X	X	X	X	X	X	9	X
5	*S. goetzeana*										-	
6	*S. grandifolia*	X	X	X	X	X	X	X	X	X	9	X
7	*S. grotei*	X	X	X	X	X		X			6	X
8	*S. inconspicua*										-	
9	*S. intermedia*	X	X	X	X	X	X				6	X
10	*S. ionantha*	X	X	X	X	X	X	X	X	X	9	X
11	*S. magungensis*	X	X	X	X	X		X	X	X	8	X
11b	*S. mag.* v. *minima*	X	X		X	X					5	X
11c	*S. mag.* v. *occidentalis*		X			X		X			3	X
12	*S. nitida*	X	X	X	X	X		X			6	X
13	*S. orbicularis*		X	X		X	X	X			5	X
13b	*S. orb.* v. *purpurea*	X	X	X	X						4	
14	*S. pendula*	X	X	X	X		X				6	X
14b	*S. pend.* v. *kizarae*	X	X		X						3	X
15	*S. pusilla*										-	
16	*S. rupicola*	X	X	X	X		X	X			6	X
17	*S. shumensis*	X	X			X	X				4	X
18	*S. teitensis*			X							1	X
19	*S. tongwensis*	X	X	X	X	X	X	X	X	X	9	X
20	*S. velutina*	X	X		X	X	X		X	X	7	X
21	*S.* sp. n. "Kacharoni"			X		X		X			3	X
22	*S.* sp. n. "Mafia Hills"	X	?								1(-2)	
23	*S.* sp. n. "Mwachi"			X		X		X			3	X
24	*S.* sp. n. "Sigi Falls"	?		X	?	X		X			3(-5)	X
25	*S.* sp. n. "Mhonda"	X									1	
26	*S.* sp. n. "Pangani Falls"	X			X						2	
	TOTAL, taxa:	**22**	**21**	**19**	**19**	**19**	**11**	**16**	**8**	**8**		**(23)**

Despite the shortcomings, the existing *ex situ* collections in European gardens represent a remarkable asset for the conservation of African violets. Only a limited amount of work is needed to transform these collections into a true gene bank, which is capable of saving most of the diversity of this genus.

This work is now under planning at Helsinki University Botanic Garden, and it is hoped that it will commence in the beginning of the year 2004. Preliminary contact has already been made with botanic gardens in Uppsala, Sweden, and Meise, Belgium, and both are willing to participate in a European *ex situ* conservation network of *Saintpaulia* (M. Lidén pers. comm.; F. Billiet pers. comm.). It is anticipated that other gardens with major holdings will be interested as well.

Botanic gardens are expected to assume a central role in the conservation of threatened plants (Anonymous 2002) and they have themselves committed to such work (Wyse Jackson & Sutherland 2000). *Ex situ* conservation of African violets in European gardens is, hence, conceptually well in line with current botanic garden strategies. An additional benefit with this particular group of plants is that they are, in practical terms, ideal subjects of this kind of work. The plants are small, easy to grow, and their vegetative propagation is quite trouble-free, which means that a comprehensive *ex situ* collection can be managed, and its genetic structure maintained, alongside every-day routines of botanic gardens. Furthermore, African violets are known and loved by thousands, which make them an ideal *ex situ* conservation object also from an educational point of view. We therefore believe *Saintpaulia* will be a prime example of *ex situ* conservation work in European botanic gardens in the near future, and we welcome all interested gardens with major holdings of wild *Saintpaulia* to do their share.

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Threatened plant collections in 'Alexandru Borza' Botanical Garden (Romania) for information and educational purposes

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Abstract. *– The paper presents the education programme of the 'Alexandru Borza' Botanical Garden. Through its collections and research aiming to clarify various aspects of the preserved species biology, as well as through its collaboration with similar organizations in the field of conservation, the 'Alexandru Borza' Botanical Garden has managed to attract a visiting public of various categories, and to organize educational activities that are appreciated by participants and are useful for all the parties involved.*

Key words : *threatened taxa, threatened plant collections, species conservation, public information, educational activities in botanical gardens.*

Introduction. The main mission of any botanical garden, that of preserving valuable species, can not be accomplished in the absence of research for the understanding of their biology. It also would certainly be meaningless if the knowledge regarding those species, biodiversity in general, and the need to protect it were not popularized. Thus, botanical gardens acquire, in addition to their attributes of research and conservation centres, that of educational centres, becoming places where the wide public can be made aware of the problems that the conservation of a species may pose.

As concluded at the Eurogard 1997 meeting in Edinburgh: "Let us take education in botanic gardens seriously. It should be given the importance – the technical status – of any other aspect of a botanical garden's scientific work" (Wyse Jackson & al. 1997).

The collections of the 'Alexandru Borza' Botanical Garden (Cluj-Napoca, Romania) include approximately 9,800 taxa cultivated in greenhouses or outdoors, in six sectors: the Ornamental (1 380), the Phytogeographical (4 300), the Systematic (3 350), the Economic sectors (220), the Nursery (475), and the sector of Endemic, Rare and Endangered plants from Romania; the latter preserves approximately 60 taxa, listed in national and international red lists and conventions (e.g. *Dianthus callizonus*, *Swertia perennis*, *Tofieldia calyculata*, *Cerastium transsilvanicum*). The institute also comprises a Botanical Museum (with approximately 7000 exhibits), a Herbarium (with more than 660,000 sheets and 325 holotypes), and a Library. This infrastructure offers a concrete and also practical background for educational activities (Micle & al. 2002).

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Aim of educational activities and practical modalities for their carrying out. Within this framework, the educational activities carried out by the 'Alexandru Borza' Botanical Garden, both in and outside it, in collaboration with partnersactive in research and conservation, non-governmental environmental organizations, researchers, teachers, had the following objectives:

- to assemble knowledge of the diversity of the plant world;
- to obtain information on the protected species as well as on the main plant formations from Romania;
- to acquire data on concrete modalities for *in situ* and *ex situ* conservation of plant species;
- to train guides for the 'Alexandru Borza' Botanical Garden;
- to multiply and distribute plant material belonging to threatened species with ornamental value.

The final aim was to make aware the participants in these activities that man, plants and the environment are permanently interdependent, as well as to form a current of opinion favourable for the development of ecological awareness.

In order to reach these aims, the collaboration with ecological schools groups from Cluj, non-governmental environmental organizations, researchers, teaching staff resulted in the organization of theoretical and practical courses by age groups, interests and training levels, both in the mentioned sectors of the Botanical Garden and at the Biological Research Institute or in the field, in the reserves and protected areas of the Cluj county (Goia & al. 1998; Şuteu & al. 2001) .

The 'Alexandru Borza' Botanical Garden greenhouses offered the possibility for live information about the diversity of exotic plants, while the sector of plants in open air offered a picture not just of the immense diversity of the plant world, but also of its evolution from organisms with an extremely simple organization to superior plants that present multiple adaptations to the environment. The newly created sector of Endemic, Rare and Endangered plants preserves one of the 32 Romanian taxa included in IUCN - Red List of Threatened Plants (1998), six of the 99 included in the list of Globally threatened plants in Europe (1998), one of the 55 listed in the Bern Convention, three of the 24 included in the Habitats Directive (Council of European Communities, 1992), and one of the 36 Romanian taxa included in the CITES List (Inskipp & Gillett 2003) (tab. 1).

Table 1. Taxa from various threatened plants lists and convention preserved in the Sector of Endemic, Rare and Endangered plants from Romania.
RLTP, IUCN-Red List of Threatened Plants; GTPE, Globally threatened plants in Europe; BC, Bern Convention; HabDir, Habitats Directive; CITES, CITES List.
VU, vulnerable; R, rare; •, present on the mentioned list.

Taxa	RLTP	GTPE	BC	HabDir	CITES
Aquilegia nigricans subsp. *subscaposa*	-	R	-	-	-
Arnica montana	-	-	-	•	-
Astragalus roemeri	VU	VU	-	-	-
Dianthus glacialis subsp. *gelidus*	-	R	-	-	-
Dianthus spiculifolius	-	R	-	-	-
Ligularia sibirica	-	-	•	•	-
Lycopodium clavatum	-	-	-	•	-
Nigritella nigra subsp. *rubra*	-	-	-	-	•
Salvia transsylvanica	-	R	-	-	-
Viola jooi	-	R	-	-	-

All the stages of territory management were presented, with emphasis on the fact that introduction of the mentioned rare species was only possible based on adequate documentation and research results of the 'Alexandru Borza' Botanical Garden on the biology of those species, the scientists of the Garden being the only ones capable of ensuring the success of the *ex situ* conservation of these valuable species (Hentea & al. 2002).

The practical importance of plants, given by their medicinal, ornamental and economic value, could be easily understood in the sectors intended for this purpose, as well as the plant species richness of Romania – 3450 species (Beldie & Moraru 1976), represented by the phytogeographical sector of the Romanian flora. The improvement of the Apuseni Mountains subsector (maintenance, repopulation with characteristic plants, placing of explanatory panels, etc.) also allowed to organize a center of information concerning the touristic potential of these mountains.

In the cell culture and plant tissue laboratory of the Biological Research Institute, the course attendants got acquainted with another *ex situ* plant conservation modality, i.e. *in vitro* cultures.

Applications in the field, in various botanical reserves, Suatu, Cheile Turzii, Zau de Câmpie, the future National Park of the Apuseni Mountains, completed the information on plant diversity, on the biotopes of these plants, which develop different adaptation strategies. In the Suatu Reserve, it could be observed how, depending on the environment in which it develops, a species may find more or less favorable conditions, and the population size of this species was evaluated for the first time (Şuteu & al. 2001).

Public awareness of environmental problems and the conservation of plant species is also raised by the organization of the 'Open Doors Days' (July 5, yearly), as well as by published illustration material, guide books and leaflets on the Botanical Garden, the Botanical Museum, and the Apuseni Mountains sector, and postcards with plants from the greenhouses and the open air sectors.

Conclusion. The impact of the educational activities was evaluated each time using questionnaires filled in by participants. The analysis of the questionnaires showed the following:

- The participants appreciated in particular their acquisition of important new information.
- In the case of pupils, especially those aged between 14-18 years, through the cooperation with the teachers, various fields of interest could be identified, which may only be approached tangentially during classes, but which can make the object of some special courses.
- The knowledge of the modalities of multiplication, *in situ* and *ex situ* species conservation, as well as the research results obtained by the 'Alexandru Borza' Botanical Garden as part of the studies on the biology of species (*Astragalus peterfii*, *Centaurea reichenbachii*, *Aquilegia nigricans* subsp. *subscaposa*, *Dianthus petraeus* subsp. *orbelicus*, *D. spiculifolius*; Hentea & al. 2004), offered a practical support for the production and distribution of plant material to those interested in growing it in their private gardens.
- The propositions and suggestions of the course attendants led to the identification of new aspects of the subjects approached, proving a good understanding of the notions taught and the awareness of pupils and the public regarding environmental issues.
- The sincere and open atmosphere of the theoretical and practical activities was appreciated.

The importance of the existence of endangered plant collections in botanical gardens not only consists in the conservation of a valuable gene fund, which allows to perform further research, but also in the fact that a scientific activity can acquire an educational role, in which case the factors involved in these fields should establish increasingly close connections.

With the here surveyed programme, the 'Alexandru Borza' Botanical Garden has succeeded in harmoniously and efficiently joining the three missions specific for the activities of any botanical garden: conservation, research, and education.

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Resolutions from EuroGard III Congress

EuroGard III, the third European Botanic Gardens Congress was held in Meise, Belgium, from 21-26 July, 2003, hosted by the National Botanic Garden of Belgium. The conference was organised by the BGCI/IABG European Botanic Gardens Consortium, and the National Botanic Garden of Belgium, together with Botanic Gardens Conservation International (BGCI) and the International Association of Botanic Gardens (IABG). The conference was attended by more than 140 delegates from botanic gardens throughout Europe.

The aims of the conference were to review the implementation of the Action Plan for Botanic Gardens in the European Union and consider the present, future and potential roles and priorities of botanic gardens in Europe in science, education, conservation, public awareness, networking, information management and dissemination, seed banking, involvement in international conventions, the efficient and ethical exchange of germplasm and in other areas.

BEDUCO II, the Second European Botanic Gardens Education Congress was organized (20-25 July, 2003) in parallel to Eurogard III

Resolution 1. – Implementation of the European Botanic Gardens Action Plan

The participants:

Reaffirmed their commitment to the implementation of the objectives of the *Action Plan for Botanic Gardens in the European Union*, and through it, agreed to work to achieve the goals of the *International Agenda for Botanic Gardens in Conservation* and the *Global Strategy for Plant Conservation;*

Welcomed the progress made by individual botanic gardens and other aligned organisations and institutions, towards the achievement of the objectives of the Action Plan since its publication in 2000, most notably in the area of networking;

Highlighted the need for urgent action to be taken by botanic gardens and other organisations to ensure the conservation of native plant diversity and their habitats in Europe, paying particular attention to species that are critically endangered;

Requested the BGCI/IABG European Botanic Gardens Consortium to include representatives from new accession countries of the European Union;

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Proposed that the Consortium should develop a series of outcome-orientated targets, milestones and indicators, with the aim of monitoring and promoting the implementation of the *Action Plan for Botanic Gardens in the European Union*, with the purpose of aligning the Action Plan with the *Global Strategy for Plant Conservation*;

Further proposed that the Consortium should make available its proposals for such targets, milestones and indicators for review by national botanic garden networks in European countries as well as by the World Botanic Gardens Congress in Barcelona, Spain (April, 2004), and subsequently to report and review progress made at EuroGard IV;

Suggested that national networks of botanic gardens in Europe should consider the development of similar national targets for their contributions to the Action Plan as well as to contribute to national plant conservation or biodiversity strategies and action plans;

Proposed that the Consortium should consider the development of proposals for the registration of projects with regional or international scope undertaken by botanic gardens that contribute to the achievement of the Action Plan, with the aim of monitoring its implementation and to act as models for further work;

Urged national botanic garden networks to review whether a similar system for the registration of projects undertaken at national level would be feasible or appropriate;

Highlighted the importance of botanic gardens and their networks promoting the implementation of the *Global Strategy for Plant Conservation* in their own countries, particularly with governments;

Urged governments, and other relevant bodies to ensure that botanic gardens have access to adequate resources to support their important work in conservation and education;

Recommended that BGCI, IABG and the European Botanic Gardens Consortium should communicate information on their activities, particularly in relation to the implementation of this resolution, to botanic garden networks and other relevant bodies throughout the world;

Encouraged botanic gardens to make information widely available through electronic and other means on their priorities, activities and achievements with the aim of raising public awareness of the importance of botanic gardens and of plant diversity;

Resolution 2. – The International Plant Exchange Network (IPEN)

Noting the framework for the work of botanic gardens in Europe that is provided by the *International Agenda for Botanic Gardens in Conservation* and the *Action Plan for Botanic Gardens in the European Union*;

Taking into account the longstanding commitment and activities of botanic gardens in the context of non-commercial exchange of plant material for research, teaching, training, education and conservation;

Noting the resolution of the 1st EASTCENTGARD Conference in Tartu, Estonia, in July 2003 which welcomed IPEN as an appropriate approach to ensuring the access and benefit sharing provisions of the Convention on Biological Diversity (CBD) are met by botanic gardens;

In order to ensure that botanic gardens continue their work effectively and ethically, according to the provisions of the CBD

The participants:

Agreed the need for joint and harmonised policies relating to the objectives of the CBD;

Pointed out the need to support measures to help increase confidence and trust between providing and recipient countries of plant material;

Endorsed IPEN as an appropriate model to meet requirements of the CBD on access to genetic resources and benefit-sharing (ABS);

Encouraged botanic gardens in the European region to join IPEN;

Urged European national network representatives to present IPEN to their respective CBD National Focal Points.

Resolution 3. – Recommendation of the BEDUCO II Congress

The participants:

Welcomed the recommendation of the Second European Botanic Gardens Education Congress (BEDUCO II), (20-25 July, 2003, Meise, Belgium) which proposed that:

- The Education and Public Awareness process should be integrated into all strategic planning for conservation, from government to individual botanic garden levels
- All forthcoming European botanic garden conferences should have unique timeslots where botanic garden managers and educators are given an opportunity to meet and discuss the implementation of common objectives included in the *Action Plan for Botanic Gardens in the European Union.*

Resolution 4. – Thanks to the Congress host

The participants:

Congratulated and thanked the National Botanic Garden of Belgium for its outstanding and generous support of the European botanic gardens in hosting the EuroGard III and BEDUCO II Congresses.

Scripta Botanica Belgica **29**

E. Robbrecht
A. Bogaerts
(eds.)

EUROGARD III

PAPERS FROM THE THIRD EUROPEAN BOTANIC GARDENS CONGRESS AND THE SECOND EUROPEAN BOTANIC GARDENS EDUCATION CONGRESS (BEDUCO II)

The volume assembles twenty-five papers from two symposia held in July, 2003 at the National Botanic Garden of Belgium. The volume includes a wide and diverse range of topics, and thus provides a valuable resource of model activities in botanic garden research, education and conservation programmes.

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